WEST Search History

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DATE: Tuesday, February 15, 2005

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L1	(method or process).clm. and (felis or pylori or helicobacter or hpylori or pyloris or pyloridis or pylorum or pylor).clm.	934
☐	L2	L1 and (il-4 or interleukin4 or interleukin-4 or il4 or interleukin).clm.	25
☐	L3	(igg2 or igg-2 or ig-g2 or (immunoglobulin near3 g2))	4540
☐	L4	felis or pylori or helicobacter or hpylori or pyloris or pyloridis or pylorum or pylor	10046
☐	L5	L4 same l3	9
		DB=EPAB; PLUR=YES; OP=AND	
☐	L6	WO-200203065-A1.did.	0
		DB=EPAB,JPAB,DWPI; PLUR=YES; OP=AND	
☐	L7	200203065	5
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L8	interferongamma or interferon-gamma or ifngamma or ifn-gamma or gamma-ifn or gammaifn or ifn	17097
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L2: Entry 5 of 25 File: PGPB Aug 12, 2004

PGPUB-DOCUMENT-NUMBER: 20040157277
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040157277 A1

TITLE: Methods for predicting and/or diagnosing the risk of gastric cancer

PUBLICATION-DATE: August 12, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Clancy, Robert Llewellyn	Newcastle		AU	
Pang, Gerald	Elizabeth Bay		AU	

APPL-NO: 10/ 695111 [PALM]
DATE FILED: October 28, 2003

RELATED-US-APPL-DATA:
Application 10/695111 is a continuation-of US application 09/979594, filed March 8, 2002, PENDING
Application 09/979594 is a a-371-of-international WO application PC/T/AU00/00441, filed May 15, 2000, UNKNOWN

INT-CL: [07] G01 N 33/574, G01 N 33/554, G01 N 33/569

US-CL-PUBLISHED: 435/007.23; 435/007.32
US-CL-CURRENT: 435/7.23; 435/7.32

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The present invention relates to methods of predicting the risk of developing cancer and in particular to a method for diagnosing, and/or predicting the risk of developing gastric cancer in a subject infected with Helicobacter.

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L2: Entry 6 of 25 File: PGPB Jul 1, 2004

PGPUB-DOCUMENT-NUMBER: 20040126356
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040126356 A1

TITLE: Compositions and methods for diagnosis and treatment of cardiovascular disorders

PUBLICATION-DATE: July 1, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Pang, Gerald	New South Wales		AU	
Conway, Patricia Lynne	New South Wales		AU	
Clancy, Robert Llewellyn	New South Wales		AU	

US-CL-CURRENT: 424/85.1

CLAIMS:

1. A method of upregulating a cytokine profile characteristic of a Th1 T-cell response relative to a cytokine profile of a Th-2 T-cell response associated with inflammation of blood vessels in a cardiovascular disorder, comprising administering to a subject in need thereof an effective amount of one or more probiotic agents for prophylaxis or treatment of the inflammation.

2. A method according to claim 1 wherein the method is a method of treating the inflammation.

3. A method according to claim 1 comprising shifting the cytokine profile characteristic of a Th2 T-cell response to a cytokine profile characteristic of a Th1 response.

4. A method according to claim 1 comprising administering a probiotic agent capable of upregulating a Th1 T-cell response and suppressing a Th2 T-cell response in the subject.

5. A method according to claim 1 comprising administering a probiotic agent capable of potentiating the action of cytokines characteristic of a Th1 T-cell response and suppressing the action of cytokines characteristic of a Th2 response in the subject.

6. A method according to claim 1 comprising administering a probiotic agent capable of upregulating a Th1 T-cell response in the subject.

7. A method according to claim 1 comprising administering a probiotic agent capable of potentiating the action of cytokines characteristic of a Th1 T-cell response in the subject.

8. A method according to claim 1 comprising administering a probiotic agent capable of suppressing a Th2 T-cell response in the subject.

9. A method according to claim 1 comprising administering a probiotic agent capable of suppressing the action of cytokines characteristic of a Th2 T-cell response in the subject.

10. A method according to claim 1 wherein the one or more probiotic agents comprises comprises a microorganism, extract or sonicate, or a mixture of some or all of the foregoing.

11. A method according to claim 10 wherein the extract comprises a cell wall fraction of the microorganism.

12. A method according to claim 11 wherein the microorganism is selected from the group consisting of yeast and bacteria.

13. A method according to claim 12 wherein the microorganism is a probiotic bacterium.

14. A method according to claim 13 wherein the probiotic bacterium is selected from the group consisting of Lactobacillus and Mycobacterium species.

15. A method according to claim 14 wherein the Lactobacillus species is capable of suppressing a Th2 response and lowering cholesterol level in the subject.

16. A method according to claim 13 wherein the probiotic bacterium is selected from Lactobacillus acidophilus, Lactobacillus fermentum, and Mycobacterium vaccae.

17. A method according to claim 12 wherein the microorganism is a bacterium selected selected from the group consisting of Lactobacillus casei, Lactobacillus plantarum, Lactobacillus chamnosus and Bifidobacterium breve.

18. A method according to claim 10 wherein the microorganism is viable.

19. A method according to claim 1 or 2 further comprising administering to the subject an effective amount of at least one pharmaceutically active agent for treating the subject in addition to the probiotic agent for up regulating a cytokine profile characteristic of a Th1 T-cell response.

20. A method according- to claim 19 wherein the pharmaceutically active agent is selected from the group consisting of lipid-lowering drugs, anti-hypertensive agents and anti-diabetic agents.

21. A method according to claim 19 wherein the probiotic agent for up regulating the the cytokine profile characteristic of the Th1 T-cell response is administered to the subject prior to, simultaneously with or subsequent to at least one pharmaceutically active agent.

22. A method according to claim 1 wherein the Th2 T-cell response associated with the disorder is exacerbated by bacterial infection, bacterial antigens, polyclonal activators, superantigens or autoantigens.

23. A method according to claim 22 wherein the infection is by, or the bacterial antigen is from, Chlamydia pneumoniae, Helicobacter pylori or non-typable Haemophilus influenzae.

24. A method according to claim 1 or 2 wherein the cardiovascular disorder is selected from stable or unstable clinical cardiovascular disease, degenerative vascular disease, atheroma and coronary artery disease.

25. A method according to claim 2-4 wherein the cardiovascular disorder is selected from the group consisting of subjects suffering from atheroma with stable or unstable clinical disease.

26. A method of diagnosing or evaluating susceptibility to inflammation of blood vessels associated with a cardiovascular disorder, comprising evaluating a T-cell response in a subject wherein an upregulated Th2 T-cell response and/or suppressed Th1 T-cell response is indicative of susceptibility to, or the presence of, the disorder.

27. A method according to claim 26 comprising determining whether the subject has an an upregulated Th2 T-cell response and a suppressed Th1 T-cell response.

28. A method according to claim 26 wherein the evaluating comprises determining whether the activity or production of one or more cytokines characteristic of the Th1 T-cell response is suppressed and/or whether the activity or production of one or more cytokines characteristic of a Th2 T-cell response is potentiated.

29. A method according to claim 28 wherein the evaluating comprises determining whether the activity or production of one or more cytokines characteristic of Th1 T-cell response is suppressed and whether the activity or production or one or more cytokines characteristic of a Th2 T-cells response is potentiated.

30. A method according to claim 28 or 29 wherein the cytokine or cytokines are selected from the group consisting of IFN-.gamma., IL-4, IL-10 and IL-12.

31. A method according to any one of claims 26 to 30 wherein the T-cell response is evaluated by analysis of circulating T-cells.

32. A method of diagnosing a cardiovascular disorder associated with inflammation of of blood vessels or evaluating whether a subject is susceptible to the inflammation, comprising: (a) measuring one or more immunoglobulin levels affected by the disorder to obtain test data; and (b) comparing the test data with reference data to evaluate whether the subject is susceptible to, or has, the inflammation, wherein the one or more immunoglobulin levels are selected from the group consisting of total immunoglobulin isotype levels and levels of total immunoglobulin isotype subclasses.

33. A method according to claim 32 comprising measuring one or more IgG levels.

34. A method according to claim 33 comprising measuring total IgG2 subclass immunoglobulin.

35. A method according to claim 33 comprising measuring the level of an IgG2 subclass specific antibody.

36. A method according to claim 33 wherein a ratio of total IgG2 subclass to IgG2 subclass specific antibody, or an altered ratio of total IgG2 subclass to IgG2 subclass specific antibody, is indicative of susceptibility to, or presence of the disorder.

37. A method according to claim 32 wherein the cardiovascular disorder is selected from subjects suffering from stable or unstable clinical cardiovascular disease,

degenerative vascular disease, coronary artery disease and atheroma.

38. A kit when used in a method of diagnosing a cardiovascular disorder or evaluating whether a subject is susceptible to the disorder, wherein the method involves measuring one or more immunoglobulin levels effected by the disorder to obtain test data, and comparing the test data with reference data to evaluate whether the subject is susceptible to, or has, the cardiovascular disorder, and wherein the kit comprises one or more reagents for performing the method together with instructions for use, and the one or more immunoglobulin levels are selected from the group consisting of total immunoglobulin isotypes and levels of total immunoglobulin isotype subclasses.

39. A kit according to claim 37 wherein the one or more reagents are selected from antibodies, buffers and control reagents.

40. A kit when used in a method of diagnosing or evaluating susceptibility to a cardiovascular disorder, wherein the method involves evaluating a T-cell response in in a subject wherein an unregulated TL2 T-cell response and/or suppressed the T-cell response is indicative of susceptibility to, or the presence of, the disorder and the kit comprises one or more reagents for performing the method together with instructions for use.

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L2: Entry 9 of 25 File: PGPB Feb 26, 2004

PGPUB-DOCUMENT-NUMBER: 20040038329
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040038329 A1

TITLE: Methods for monitoring treatment of helicobacter infection and for predicting predicting the likelihood of successful eradication

PUBLICATION-DATE: February 26, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Clancy, Robert Llewellyn	New South Wales		AU	
Borody, Thomas Julius	New South Wales		AU	
Pang, Gerarld	New South Wales		AU	
Ren, Zhigang	New South Wales		AU	

US-CL-CURRENT: 435/30; 424/164.1, 424/234.1, 424/93.4, 435/34

CLAIMS:

The claims defining the invention are as follows:

1. A method of monitoring eradication of Helicobacter infection in a subject treated treated for the infection, including: i) determination of IgG2 anti-H. pylori antibody level in a saliva sample; ii) comparison of the IgG2 anti-H. pylori antibody level with a predetermined control IgG2 anti-H. pylori antibody level, wherein a reduction in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates eradication of Helicobacter.

2. A method of monitoring efficacy of treatment of Helicobacter infection in a subject treated for the infection, including: i) determination of IgG2 anti-H. pylori antibody level in a saliva sample; ii) comparison of the IgG2 anti-H. pylori antibody level with a predetermined control IgG2 anti-H. pylori antibody level, wherein a reduction in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates efficacious treatment of Helicobacter.

3. A method of monitoring relapse or reinfection with Helicobacter in a subject treated for infection with Helicobacter, including: i) determination of IgG2 anti-H. H. pylori antibody level in a saliva sample; ii) comparison of the IgG2 anti-H. pylori antibody level with a predetermined control IgG2 anti-H. pylori antibody level, wherein an increase in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates relapse or reinfection with Helicobacter.

4. A method of detecting unresponsiveness of a subject to treatment of Helicobacter infection, including: (i) determination of IgG2 anti-H. pylori antibody level in a saliva sample; (ii) comparison of the IgG2 anti-H. pylori antibody level with a

predetermined control IgG2 anti-H. pylori antibody level, wherein lack of change in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates lack of response to treatment.

5. A method according to any one of claims 1 to 4, wherein the IgG2 anti-H. pylori antibody is detected by an immunoassay.

6. A method according to claim 5, wherein the assay is ELISA.

7. A method according to any one of claims 1 to 6, wherein the control levels of IgG2 anti-H. pylori antibody is established in samples of saliva obtained from subjects not infected by H. pylori.

8. A method according to any one of claims 1 to 6, wherein the control levels of IgG2 anti-H. pylori antibody are determined in subject's own saliva sample.

9. A kit for monitoring treatment of Helicobacter infection, including, (i) Helicobacter antigen (ii) reagent for determining IgG2 subclass antibody.

10. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination of IL-4 level in a sample from the subject; (ii) comparison of the the IL-4 level with a predetermined control or standard IL-4 level, (iii) wherein a level of IL-4 in the sample from the subject above the control or standard IL-4 level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard IL-4 level is predictive of the likelihood of eradication failure.

11. A method according to claim 10 wherein the sample is a blood sample.

12. A method according to claim 10 or claim 11, wherein the IL-4 is detected by an immunoassay.

13. A method according to claim 12, wherein the assay is ELISA.

14. A method according to any one of claims 10 to 13, wherein the control or standard level of IL-4 is established from analysis of samples obtained from subjects not infected by H. pylori and/or subjects having successfully eradicated H. H. pylori and/or subjects infected by H. pylori.

15. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination of interferon-.gamma. (INF-.gamma.) level in a sample from the subject; (ii) comparison of the INF-.gamma. level with a predetermined control or standard INF-.gamma. level, (iii) wherein a level of INF-.gamma. in the sample from the subject below the control or standard INF-.gamma. level is predictive of the likelihood of successful eradication and a level of INF-.gamma. above the control or standard level is predictive of the likelihood of eradication failure.

16. A method according to claim 15 wherein the sample is a blood sample.

17. A method according to claim 15 or claim 16, wherein the IFN-.gamma. level is detected by an immunoassay.

18. A method according to claim 17, wherein the assay is ELISA.

19. A method according to any one of claims 15 to 18, wherein the control or standard level of IFN-.gamma. is established from analysis of samples obtained from subjects not infected by H. pylorii and/or subjects having successfully eradicated H. pylori. and/or subjects infected by H. pylori.

20. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination of immunoglobulin G (IgG) level in a sample from the subject; (ii) comparison of the IgG level with a predetermined control or standard IgG level, (iii) wherein a level of IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

21. A method according to claim 20 wherein the sample is a serum sample.

22. A method according to claim 20 wherein the sample is a saliva sample.

23. A method according to any one of claims 20 to 22, wherein the control or standard level of IgG is established from analysis of samples obtained from subjects not infected by H. pylori and/or subjects having successfully eradicated H. H. pylori and/or subjects infected by H. pylori.

24. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination a combination of IL-4 and/or INF-.gamma. and/or IgG levels in a sample from the subject; (ii) comparison of the IL-4 and/or INF-.gamma. and/or IgG levels with a predetermined control or standard L-4 and/or IF-.gamma. and/or IgG level respectively, wherein a level of IL-4 in the sample from the subject above the the control or standard level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard level is predictive of of the likelihood of eradication failure, and wherein a level of INF-.gamma. in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IFN-.gamma. above the control or standard level is predictive of the likelihood of eradication failure, and wherein a level of IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

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L2: Entry 10 of 25 File: PGPB Feb 12, 2004

PGPUB-DOCUMENT-NUMBER: 20040029259
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040029259 A1

TITLE: Method and system for the detection of cardiac risk factors

PUBLICATION-DATE: February 12, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
McDevitt, John T.	Austin	TX	US	
Anslyn, Eric V.	Austin	TX	US	
Shear, Jason B.	Austin	TX	US	
Neikirk, Dean P.	Austin	TX	US	
Christodoulides, Nick J.	Austin	TX	US	

APPL-NO: 10/ 427744 [PALM]
DATE FILED: April 28, 2003

RELATED-US-APPL-DATA:
Application is a non-provisional-of-provisional application 60/375775, filed April 26, 2002,

INT-CL: [07] C12 M 1/34

US-CL-PUBLISHED: 435/287.2
US-CL-CURRENT: 435/287.2

REPRESENTATIVE-FIGURES: 1

ABSTRACT:

A system for the rapid characterization of multi-cardiovascular risk factor analyte fluids, in one embodiment, includes a light source, a sensor array, and a detector. The sensor array is formed from a supporting member, in which a plurality of cavities may be formed. A series of chemically sensitive particles, in one embodiment, are positioned within the cavities. The particles may produce a signal when a receptor, coupled to the particle, interacts with the cardiovascular risk factor analyte and the particle-analyte complex is visualized using a visualization reagent. Using pattern recognition techniques, the analytes within a multi-analyte fluid may be characterized. In an embodiment, each cavity of the plurality of cavities is designed to capture and contain a specific size particle. Flexible projections may be positioned over each of the cavities to provide retention of the particles in the cavities.

PRIORITY CLAIM

[0001] This application claims priority to Provisional Patent Application No. 60/375,775 entitled "METHOD AND SYSTEM FOR THE DETECTION OF CARDIAC RISK FACTORS" filed on Apr. 26, 2002.

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11555037 PMID: 8867497

Enhanced T-helper 2 lymphocyte responses: immune mechanism of Helicobacter pylori infection.

Fan X G; Yakoob J; Fan X J; Keeling P W

Department of Clinical Medicine, St. James's Hospital, Dublin.

Irish journal of medical science (IRELAND) Jan-Mar 1996, 165 (1) p37-9, ISSN 0021-1265 Journal Code: 7806864

Publishing Model Print

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Several lines of evidence implicate **Helicobacter pylori** (H. **pylori**) infection in gastroduodenal inflammation. However, the exact pathogenesis of H. **pylori** infection is not fully understood. T-helper (TH) lymphocytes may be subdivided into TH1 and TH2 cells based on the distinct patterns of cytokine production. TH1 reaction is associated with immunity or resistance to infection, while TH2 reaction is associated with the progression or persistence of infection. The production of **interferon** -gamma (INF-gamma) and interleukin 2 (IL-2), which are type 1 cytokines, is decreased in H. **pylori** infection. Enhanced production of type 2 cytokines (IL-4) and IL-6) is observed in individuals with H. **pylori** infection. Suppressed proliferative responses of peripheral blood and gastric lymphocytes have also been demonstrated in patients with H. **pylori** colonisation, suggesting that specific T-cell responses may be down-regulated by an enhanced TH2 reaction. Suppressed TH1 and enhanced TH2 responses in H. **pylori** infection may be involved in the immunopathogenesis of chronic H. **pylori** infection. (30 Refs.)

Descriptors: *Gastrointestinal Diseases--microbiology--MI; *Helicobacter Infections--immunology--IM; *Helicobacter pylori--immunology--IM; *T-Lympho cytes, Helper-Inducer--metabolism--ME; Chronic Disease; Gastrointestinal Diseases--immunology--IM; Gastrointestinal Diseases--physiopathology--PP; Helicobacter Infections--physiopathology--PP; Humans; Interferon-alpha --biosynthesis--BI; Interleukin-2--biosynthesis--BI

CAS Registry No.: 0 (Interferon-alpha); 0 (Interleukin-2)

Record Date Created: 19961203

Record Date Completed: 19961203

12763557 PMID: 10695551

Role of Helicobacter **pylori infection in extragastroduodenal disorders: introductory remarks.**

Konturek S J; Konturek P C; Pieniazek P; Bielanski W

Department of Physiology, Jagiellonian University School of Medicine, Cracow, Poland. mpogonow@cyf-kr.edu.pl

Journal of physiology and pharmacology - an official journal of the Polish Physiological Society (POLAND) Dec 1999, 50 (5) p683-94, ISSN 0867-5910 Journal Code: 9114501

Publishing Model Print

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Numerous studies initiated by Warren and Marshall in 1982 confirmed the crucial role of H. pylori infection in the pathogenesis of gastritis, peptic ulcer and possibly also gastric cancer leading to reappraisal of fundamental concept of gastric pathophysiology. These topics were covered, in part, by our previous H. pylori-related symposium I (1995), II (1997) and III (1999) organized in Cracow. H. pylori is one of the most frequent causes of gastroduodenal infection worldwide, resulting in the release of various bacterial and host dependent cytotoxic substances including ammonia, platelet activating factor (PAF), cytotoxins and lipopolysaccharides (LPS) as well as cytokines such as interleukins (IL)-1-12, tumor necrosis factor alpha (TNF(alpha), interferon gamma (**INFgamma**) and reactive oxygen species (ROS). Recently, several extradigestive pathologies have been linked to H. pylori infection including cardiovascular, cutaneous, autoimmune, esophageal and other diseases such as sideropenic anemia, growth retardation, extragastric MALT-lymphoma etc. The potential role of H. pylori infection in the pathogenesis of these extradigestive disorders has been based on facts that 1) local gastric inflammation may exert systemic effects, 2) chronic infection of gastric mucosa induces immune responses that are able to cause the lesions remote to primary site of infection and 3) H. pylori eradication improves the extradigestive disorders. The aim of present III International Symposium is to provide critical reviews based on personal experience and the available literature about extragastric manifestations of H. pylori infection. The ultimate goal of this symposium is to foster interdisciplinary research and exchange of opinion about the possible involvement of H. pylori in extradigestive pathologies. (53 Refs.)

Descriptors: ***Helicobacte** r Infections; * **Helicobacter** pylori; Animals; Autoimmune Diseases--microbiology--MI; Biliary Tract Diseases--microbiology --MI; Gastroesophageal Reflux--microbiology--MI; **Helicobacter** Infections --immunology--IM; Humans; Liver Diseases--microbiology--MI; Vascular Diseases--microbiology--MI

Record Date Created: 20000328

Record Date Completed: 20000328

Classification Codes: 002A05B10; 002A06C05A

17/9/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12062525 PMID: 9358084

Helicobacter pylori induces proinflammatory cytokines and major histocompatibility complex class II antigen in mouse gastric epithelial cells.

Maekawa T; Kinoshita Y; Matsushima Y; Okada A; Fukui H; Waki S; Kishi K; Kawanami C; Nakata H; Hassan S; Wakatsuki Y; Ota H; Amano K; Nakao M; Chiba T

Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, Japan.

Journal of laboratory and clinical medicine (UNITED STATES) Oct 1997, 130 (4) p442-9, ISSN 0022-2143 Journal Code: 0375375

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

Although Helicobacter pylori has been reported to stimulate the release of various cytokines from gastric tissue, it remains unknown whether normal and nontumorous gastric epithelial cells produce these cytokines. Therefore, in this study, we used a normal mouse gastric surface mucous cell line (GSM06) to determine whether gastric epithelial cells produce proinflammatory cytokines in response to H. pylori. The expression of MHC class II antigen was also examined, to investigate whether gastric epithelial cells participate in the immune response to H. pylori. In the study, GSM06 cells were incubated with H. pylori or its lipopolysaccharide (LPS). Proinflammatory cytokines were detected by Northern and Western blot analysis. The expression of MHC class II antigen was examined by fluorescence activated cell sorter (FACS) analysis. Genetic expression of proinflammatory cytokines such as interleukin-1alpha, tumor necrosis factor-alpha, and cytokine-induced neutrophil chemoattractant-2beta was enhanced by both intact and sonicated H. pylori, but not by H. pylori LPS. The expression of MHC class II antigen was induced by H. pylori more strongly than by interferon-gamma. We conclude that H. pylori induces the expression of proinflammatory cytokines and MHC class II antigen in gastric epithelial cells. Gastric epithelial cells may act as antigen-presenting cells and participate in the immune response to H. pylori infection.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Chemokines, CXC; *Cytokines--biosynthesis--BI; *Gastric Mucosa--immunology--IM; *Gastric Mucosa--microbiology--MI; *Helicobacter pylori--physiology--PH; *Histocompatibility Antigens Class II--biosynthesis --BI; *Intercellular Signaling Peptides and Proteins; Animals; Cell Line; Chemotactic Factors--biosynthesis--BI; Chemotactic Factors--genetics--GE; Cytokines--genetics--GE; Escherichia coli; Flow Cytometry; Gene Expression; Growth Substances--biosynthesis--BI; Growth Substances--genetics--GE; Helicobacter **pylori** --immunology--IM; Histocompatibility Antigens Class II --genetics--GE; Histocompatibility Antigens Class II--immunology--IM; Histocytochemistry; Inflammation; Interferon Type II--pharmacology--PD; Interleukin-1--biosynthesis--BI; Interleukin-1--genetics--GE; Kinetics; Lipopolysaccharides--pharmacology--PD; Mice; Polymyxin B--pharmacology--PD; RNA, Messenger--genetics--GE; RNA, Messenger--metabolism--ME; Tumor Necrosis Factor-alpha--biosynthesis--BI; Tumor Necrosis Factor-alpha --genetics--GE

CAS Registry No.: 0 (Chemokines, CXC); 0 (Chemotactic Factors); 0 (Cxcl1 protein, mouse); 0 (Cytokines); 0 (Growth Substances); 0 (Histocompatibility Antigens Class II); 0 (Intercellular Signaling

rapid urease test. H pylori antigen was capable of stimulating peripheral blood lymphocyte proliferative responses even in H **pylori** negative patients. Peripheral blood lymphocyte proliferative responses to H pylori (but not to purified protein derivative or phythaemagglutinin) were significantly lower in H pylori positive than H pylori negative patients. Similarly, antigen specific proliferative responses and interferon gamma production by gastric lamina propria lymphocytes were also depressed in H pylori positive patients compared with H pylori negative patients. CD8 and CD22 positive lamina propria lymphocytes were increased in H pylori positive patients. These data show that antigen specific responses to H pylori are significantly lower in H pylori positive patients and could indicate activation of antigen specific suppression.

Tags: Female; Male

Descriptors: *Helicobacter Infections--immunology--IM; *Helicobacter pylori--immunology--IM; *Lymphocyte Activation; *Stomach--immunology--IM; *T-Lymphocytes--immunology--IM; Adolescent; Adult; Aged; Antigen-Presenting Cells--immunology--IM; Antigens, Bacterial--immunology--IM; Cells, Cultured ; Cytokines--metabolism--ME; Helicobacter Infections--blood--BL; Humans; Immunophenotyping; Interferon Type II--biosynthesis--BI; Interleukin-2 --pharmacology--PD; Middle Aged; T-Lymphocytes--cytology--CY; T-Lymphocytes--metabolism--ME

CAS Registry No.: 0 (Antigens, Bacterial); 0 (Cytokines); 0 (Interleukin-2); 82115-62-6 (Interferon Type II)

Record Date Created: 19941220

Record Date Completed: 19941220

17/9/8 (Item 8 from file: 159)

DIALOG(R)File 159:Cancerlit

02049582 94131779 PMID: 8300378

Interferon-gamma and tumour necrosis factor production in patients with Helicobacter pylori infection.

Fan X J; Chua A; O'Connell M A; Kelleher D; Keeling P W

Department of Clinical Medicine, St. James's Hospital, Trinity College Dublin, Ireland.

Ir J Med Sci (IRELAND) Oct 1993, 162 (10) p408-11, ISSN 0021-1265 Journal Code: 7806864

Document Type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Subfile: INDEX MEDICUS

The production of the cytokines, interferon-gamma and tumour necrosis factor by human antral mucosa cells and stimulated peripheral blood mononuclear cells were determined by enzyme linked immunosorbent assay and L929 bioassay respectively. Tumour necrosis factor production by peripheral blood mononuclear cells in response to Helicobacter pylori stimulation was depressed in Helicobacter pylori positive individuals, compared to Helicobacter pylori negative individuals ($P < 0.05$). There was no difference in tumour necrosis factor production by peripheral blood mononuclear cells in response to purified protein **derivative** . However, tumour necrosis factor production by cells isolated from gastric mucosa during short term culture was significantly higher in Helicobacter pylori positive patients ($P < 0.05$) than negative patients, indicating a probable macrophage response. Levels of interferon-gamma did not differ significantly in the gastric explant culture from the two groups. The results show that Helicobacter pylori negative patients have a stronger peripheral cellular immune response to Helicobacter pylori infection. The higher levels of tumour necrosis factor production by antral mucosa cells in Helicobacter pylori positive patients may reflect the infiltration of T lymphocytes and macrophages within the local mucosa.

Tags: Female; Human; Male

Major Descriptors: *Gastritis--immunology--IM; *Helicobacter Infections --immunology--IM; *Helicobacter pylori--immunology--IM; *Interferon Type II --biosynthesis--BI; *Tumor Necrosis Factor--biosynthesis--BI

Veterans Affairs Wadsworth Medical Service, Los Angeles, California, USA.
American journal of physiology (UNITED STATES) May **1996** , 270 (5 Pt 1) pG783-8, ISSN 0002-9513 Journal Code: 0370511
Contract/Grant No.: DK-17294; DK; NIDDK; DK-3101; DK; NIDDK
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Patients with **Helicobacter pylori** -associated gastritis have an increased release of gastrin. The mechanisms by which H. **pylori** affects the endocrine cells are unclear. We have used primary cultures containing canine antral G cells to examine the effects of human blood mononuclear cells, purified monocytes and lymphocytes, recombinant cytokines, and NH4Cl on gastrin release. Mononuclear cells and purified monocytes in direct contact with G cells stimulated gastrin release dose dependently. Separating mononuclear cells from G cells by Transwell filters with 0.4-micron pore size still produced a significant increase of gastrin release. Three human recombinant cytokines, interferon-gamma, tumor necrosis factor-alpha, and interleukin-2, but not interleukin-6 and interleukin-1 beta, each produced dose-dependent increases of gastrin stimulation. NH4Cl did not stimulate gastrin release. We conclude that mononuclear cells and purified monocytes prepared from human blood, as well as several cytokines, stimulate gastrin release from antral G cells. These factors may play an important role in the pathogenesis of H. **pylori** -associated hypergastrinemia.

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Cytokines--pharmacology--PD; *Gastrins--metabolism--ME; *Monocytes--physiology--PH; *Pyloric Antrum--metabolism--ME; Animals; Cells, Cultured; Dogs; Humans; Pyloric Antrum--cytology--CY; Pyloric Antrum--drug effects--DE; Recombinant Proteins

CAS Registry No.: 0 (Cytokines); 0 (Gastrins); 0 (Recombinant Proteins)

Record Date Created: 19961206
Record Date Completed: 19961206

7/9/31
DIALOG(R)File 155:MEDLINE(R)

11217475 PMID: 8557346

Role of the host in pathogenesis of Helicobacter **-associated gastritis: H. felis infection of inbred and congenic mouse strains.**

Mohammadi M ; Redline R; Nedrud J; Czinn S

Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106, USA.

Infection and immunity (UNITED STATES) Jan **1996** , 64 (1) p238-45, ISSN 0019-9567 Journal Code: 0246127
Contract/Grant No.: DK-46461; DK; NIDDK; HL-37117; HL; NHLBI
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

In humans, **Helicobacter pylori** establishes a chronic infection which can result in various degrees of gastric inflammation, peptic ulcer disease, and a predisposition to gastric cancer. It has been suggested that bacterial virulence factors such as the vacuolating toxin (VacA) and the cytotoxin-associated gene product (CagA) may play a major role in determining the clinical outcome of **Helicobacter** infections. The role of host responses in these varied outcomes has received little attention. **Helicobacter** felis, which does not express CagA or VacA, causes chronic infection and inflammation in a well-characterized mouse model. We have

11535832 PMID: 8844467

Cytokine gene expression in the gastric mucosa: its role in chronic gastritis.

Ishihara S; Fukuda R; Fukumoto S

Second Department of Internal Medicine, Shimane Medical University, Japan.

Journal of gastroenterology (JAPAN) Aug 1996, 31 (4) p485-90, ISSN 0944-1174 Journal Code: 9430794

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

There have been few studies of cytokine expression in the gastric mucosa of patients with chronic gastritis. In the present study, to elucidate the expression of cytokines in the gastric mucosa and the immunopathological roles played by these cytokines in chronic gastritis, we investigated cytokine gene expression, by reverse transcription polymerase chain reaction, in gastric biopsy specimens obtained from 29 endoscopically normal patients with chronic gastritis. The cytokines examined and the mRNA positivity were: interleukin (IL)-1 beta (21%), IL-2 (0%), IL-3 (7%), IL-4 (41%), IL-5 (17%), IL-6 (53%), IL-8 (98%), **interferon** gamma (IFN-gamma) (69%), and tumor necrosis factor alpha (TNF-alpha) (24%). Although the histological severity of the gastritis was closely associated with **Helicobacter pylori** infection, the positivities of these cytokine mRNAs did not show a relationship with either H. **pylori** infection or with histological inflammation. Our findings suggest that the gastric mucosa responds to all exogenous antigens, including H. **pylori** , in the same fashion immunologically, and that these cytokines do not contribute to the induction of inflammation associated with H. **pylori** infection.

Tags: Female; Male

Descriptors: *Gastric Mucosa--metabolism--ME; *Gastritis--genetics--GE; *Interferon Type II--biosynthesis--BI; *Interleukins--biosynthesis--BI; *Tumor Necrosis Factor-alpha--biosynthesis--BI; Gastritis--metabolism--ME; Gastritis--microbiology--MI; Gene Expression; Helicobacter Infections --diagnosis--DI; **Helicobacter** Infections--metabolism--ME; **Helicobacter pylori** --isolation and purification--IP; Humans; **Interferon** Type II --genetics--GE; Interleukins--genetics--GE; Middle Aged; Polymerase Chain Reaction; RNA, Messenger--genetics--GE; Tumor Necrosis Factor-alpha --genetics--GE

CAS Registry No.: 0 (Interleukins); 0 (RNA, Messenger); 0 (Tumor Necrosis Factor-alpha); 82115-62-6 (Interferon Type II)

Record Date Created: 19961223

Record Date Completed: 19961223

10915420 PMID: 7698689

Interferon gamma and interleukin 4 secreting cells in the gastric antrum in Helicobacter pylori positive and negative gastritis.

Karttunen R; Karttunen T; Ekre H P; MacDonald T T

Department of Paediatric Gastroenterology, St Bartholomew's Hospital, London.

Gut (ENGLAND) Mar 1995, 36 (3) p341-5, ISSN 0017-5749

Journal Code: 2985108R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

Little is known of the function of the T cells in the inflammatory infiltrate in **Helicobacter** pylori associated gastritis. This study thus measured T cell in vivo activation by enumerating the frequency of interferon gamma (IFN gamma) and interleukin 4 (IL 4) secreting cells isolated from the gastric antral mucosa in patients with or without gastritis and in H pylori positive and negative gastritis. Fifty four samples were examined for cytokine secretion. Four antral biopsy specimens from each patient (n = 51) were taken during diagnostic endoscopy. One was used to estimate histological gastritis and the presence of H pylori, and three of the samples were used to isolate T cells by enzymatic digestion. IFN gamma and IL 4 secreting cells were enumerated by ELISPOT. Thirty four samples had gastritis and 79% of those were H pylori positive. None of the samples from non-inflamed mucosa had H pylori. The numbers of IFN gamma secreting cells per 10(5) T cells were higher in gastritis than in normal mucosa (145 v 20 IFN gamma spots, $p < 0.01$), and higher in H pylori negative than H pylori positive gastritis (371 v 110 IFN gamma spots, $p < 0.05$). The frequencies of IL 4 secreting cells did not differ between gastritis and non-inflamed mucosa. In conclusion, there is an increase in IFN gamma secreting cells but not in IL 4 secreting cells in H pylori positive and negative gastritis. It is not known if this TH1 type reaction has a pathogenetic or protective role.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Gastritis--pathology--PA; *Helicobacter Infections --pathology--PA; *Helicobacter **pylori** ; *Interferon Type II--secretion--SE ; *Interleukin-4--secretion--SE; *Pyloric Antrum--secretion--SE; Adult; Aged; Aged, 80 and over; Cell Division; Gastritis--immunology--IM; Helicobacter Infections--immunology--IM; Humans; Lymphocyte Count; Middle Aged; Pyloric Antrum--pathology--PA; T-Lymphocytes--pathology--PA

CAS Registry No.: 207137-56-2 (Interleukin-4); 82115-62-6 (Interferon Type II)

Record Date Created: 19950504

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B 155
02mar05 16:24:53 User228206 Session D2375.1
$0.00 0.187 DialUnits FileHomeBase
$0.00 Estimated cost FileHomeBase
$0.02 INTERNET
$0.02 Estimated cost this search
$0.02 Estimated total session cost 0.187 DialUnits

File 155:MEDLINE(R) 1951-2005/Feb W4
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Please see HELP NEWS 154.

Set	Items	Description

?

Ref	Items	RT	Index-term
E1	46374		FLOW //REGIONAL BLOOD (REGIONAL BLOOD FLOW)
E2	215		FLOW //RENAL PLASMA (RENAL PLASMA FLOW)
E3	52727	8	*FLOW CYTOMETRY
E4	4		FLOW CYTOMETRY --ADVERSE EFFECTS --AE
E5	2		FLOW CYTOMETRY --CLASSIFICATION --CL
E6	36		FLOW CYTOMETRY --ECONOMICS --EC
E7	1		FLOW CYTOMETRY --ETHICS --ES
E8	20		FLOW CYTOMETRY --HISTORY --HI
E9	908		FLOW CYTOMETRY --INSTRUMENTATION --IS
E10	6524		FLOW CYTOMETRY --METHODS --MT
E11	1		FLOW CYTOMETRY --NURSING --NU
E12	381		FLOW CYTOMETRY --STANDARDS --ST

Enter P or PAGE for more
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Ref    Items   RT  Index-term
E13      125       FLOW CYTOMETRY --STATISTICS AND NUMERICAL DATA
E14       50       FLOW CYTOMETRY --TRENDS --TD
E15        4       FLOW CYTOMETRY --UTILIZATION --UT
E16      606       FLOW CYTOMETRY --VETERINARY --VE
E17      981    1  FLOW INJECTION ANALYSIS
E18        2       FLOW INJECTION ANALYSIS --ECONOMICS --EC
E19      194       FLOW INJECTION ANALYSIS --INSTRUMENTATION --IS
E20      402       FLOW INJECTION ANALYSIS --METHODS --MT
E21       13       FLOW INJECTION ANALYSIS --STANDARDS --ST
E22       12       FLOW INJECTION ANALYSIS --STATISTICS AND NUMER
E23        4       FLOW INJECTION ANALYSIS --TRENDS --TD
E24        2       FLOW INJECTION ANALYSIS --VETERINARY --VE

          Enter P or PAGE for more
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Ref    Items   RT  Index-term
E25        0    1  FLOW MICROFLUORIMETRY
E26      888       FLOW  RATE  //MAXIMAL EXPIRATORY (MAXIMAL EXPIRATORY FLOW
                   RATE)
E27      749       FLOW  RATE //MAXIMAL MIDEXPIRATORY (MAXIMAL MIDEXPIRATORY
                   FLOW RATE)
E28     4146       FLOW RATE //PEAK EXPIRATORY (PEAK EXPIRATORY FLOW RATE)
E29     1754       FLOW  RATES  //FORCED  EXPIRATORY (FORCED EXPIRATORY FLOW
                   RATES)
E30    35808       FLOW VELOCITY //BLOOD (BLOOD FLOW VELOCITY)
E31      762       FLOW-VOLUME    CURVES   //MAXIMAL   EXPIRATORY   (MAXIMAL
                   EXPIRATORY FLOW-VOLUME CURVES)
E32       35       FLOW,   EFFECTIVE   //RENAL   BLOOD   (RENAL  BLOOD  FLOW,
                   EFFECTIVE)
E33      148       FLOW,   EFFECTIVE  //RENAL  PLASMA  (RENAL  PLASMA  FLOW,
                   EFFECTIVE)
E34        0    1  FLOW, PULSATING
E35        1       FLOWA
E36        1       FLOWABILITIES

          Enter P or PAGE for more
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S E1-E24
          46374  FLOW //REGIONAL BLOOD  (REGIONAL BLOOD FLOW)
            215  FLOW //RENAL PLASMA  (RENAL PLASMA FLOW)
          52727  FLOW CYTOMETRY
              4  FLOW CYTOMETRY --ADVERSE EFFECTS --AE
              2  FLOW CYTOMETRY --CLASSIFICATION --CL
             36  FLOW CYTOMETRY --ECONOMICS --EC
              1  FLOW CYTOMETRY --ETHICS --ES
             20  FLOW CYTOMETRY --HISTORY --HI
            908  FLOW CYTOMETRY --INSTRUMENTATION --IS
           6524  FLOW CYTOMETRY --METHODS --MT
              1  FLOW CYTOMETRY --NURSING --NU
            381  FLOW CYTOMETRY --STANDARDS --ST
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             50  FLOW CYTOMETRY --TRENDS --TD
              4  FLOW CYTOMETRY --UTILIZATION --UT
            606  FLOW CYTOMETRY --VETERINARY --VE
            981  FLOW INJECTION ANALYSIS
              2  FLOW INJECTION ANALYSIS --ECONOMICS --EC
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             194  FLOW INJECTION ANALYSIS --INSTRUMENTATION --IS
             402  FLOW INJECTION ANALYSIS --METHODS --MT
              13  FLOW INJECTION ANALYSIS --STANDARDS --ST
              12  FLOW INJECTION ANALYSIS --STATISTICS AND NUMER
               4  FLOW INJECTION ANALYSIS --TRENDS --TD
               2  FLOW INJECTION ANALYSIS --VETERINARY --VE
      S1  100271  E1-E24
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Ref    Items Type  RT  Index-term
R1     52727        8 *FLOW CYTOMETRY
R2     52727   X      DC=E5.196.712.516.600.240.350. (FLOW CYTOMETRY)
R3     52727   X      DC=E5.200.500.386.350. (FLOW CYTOMETRY)
R4         0   X    1  CYTOFLUOROMETRY, FLOW
R5         0   X    1  CYTOMETRY, FLOW
R6         0   X    1  FLOW MICROFLUORIMETRY
R7         0   X    1  FLUORESCENCE-ACTIVATED CELL SORTING
R8         0   X    1  MICROFLUOROMETRY, FLOW
R9      1594   B    8  CYTOPHOTOMETRY
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S R1:R9
      S2   54151  R1:R9
?


Ref    Items   RT  Index-term
E1         2       HELICOBACETER
E2         2       HELICOBACTEER
E3     20417    9 *HELICOBACTER
E4        18       HELICOBACTER --CHEMISTRY --CH
E5       147       HELICOBACTER --CLASSIFICATION --CL
E6         7       HELICOBACTER --CYTOLOGY --CY
E7        28       HELICOBACTER --DRUG EFFECTS --DE
E8        38       HELICOBACTER --ENZYMOLOGY --EN
E9       197       HELICOBACTER --GENETICS --GE
E10       32       HELICOBACTER --GROWTH AND DEVELOPMENT --GD
E11       71       HELICOBACTER --IMMUNOLOGY --IM
E12      267       HELICOBACTER --ISOLATION AND PURIFICATION --IP

          Enter P or PAGE for more
?


Ref    Items   RT  Index-term
E13       30       HELICOBACTER --METABOLISM --ME
E14       86       HELICOBACTER --PATHOGENICITY --PY
E15       27       HELICOBACTER --PHYSIOLOGY --PH
E16        1       HELICOBACTER --RADIATION EFFECTS --RE
E17       56       HELICOBACTER --ULTRASTRUCTURE --UL
E18       16    3  HELICOBACTER FELIS
E19        1       HELICOBACTER FELIS --CLASSIFICATION --CL
E20        1       HELICOBACTER FELIS --DRUG EFFECTS --DE
E21        1       HELICOBACTER FELIS --GENETICS --GE
E22        5       HELICOBACTER FELIS --IMMUNOLOGY --IM
E23        1       HELICOBACTER FELIS --ISOLATION AND PURIFICATIO
E24        4       HELICOBACTER FELIS --PATHOGENICITY --PY

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Ref    Items   RT  Index-term
E25       58    6  HELICOBACTER HEILMANNII
E26        4       HELICOBACTER HEILMANNII --CLASSIFICATION --CL
E27        1       HELICOBACTER HEILMANNII --CYTOLOGY --CY
E28        1       HELICOBACTER HEILMANNII --DRUG EFFECTS --DE
E29        6       HELICOBACTER HEILMANNII --GENETICS --GE
E30        2       HELICOBACTER HEILMANNII --GROWTH AND DEVELOPME
E31        3       HELICOBACTER HEILMANNII --IMMUNOLOGY --IM
E32       38       HELICOBACTER HEILMANNII --ISOLATION AND PURIFI
E33        2       HELICOBACTER HEILMANNII --METABOLISM --ME
E34        9       HELICOBACTER HEILMANNII --PATHOGENICITY --PY
E35        4       HELICOBACTER HEILMANNII --ULTRASTRUCTURE --UL
E36       11    3  HELICOBACTER HEPATICUS

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S HELICOBACTER?
      S3   20426  HELICOBACTER?
?

S PYLORI?
      S4   32083  PYLORI?
?


Set     Items   Description
S1     100271   E1-E24
S2      54151   R1:R9
S3      20426   HELICOBACTER?
S4      32083   PYLORI?
?

S (S1 OR S2) (50N) (S3 OR S4)
          100271  S1
           54151  S2
           20426  S3
           32083  S4
      S5      55  (S1 OR S2) (50N) (S3 OR S4)
?

S S5/2000:2005
              55  S5
         2777067  PY=2000 : PY=2005
      S6      25  S5/2000:2005
?

S S5 NOT S6
              55  S5
              25  S6
      S7      30  S5 NOT S6
?


Set     Items   Description
S1     100271   E1-E24
S2      54151   R1:R9
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S3      20426   HELICOBACTER?
S4      32083   PYLORI?
S5         55   (S1 OR S2) (50N) (S3 OR S4)
S6         25   S5/2000:2005
S7         30   S5 NOT S6
?

T S7/9/ALL
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7/9/1

DIALOG(R)File 155:MEDLINE(R)

13548749 PMID: 10520838

Eradication of Helicobacter pylori normalizes elevated mucosal levels of epidermal growth factor and its receptor.

Coyle W J; Sedlack R E; Nemec R; Peterson R; Duntemann T; Murphy M; Lawson J M

Department of Gastroenterology, Naval Medical Center, Portsmouth, Virginia 23708, USA.

American journal of gastroenterology (UNITED STATES) Oct 1999, 94 (10) p2885-9, ISSN 0002-9270 Journal Code: 0421030

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

OBJECTIVE: Helicobacter pylori (H. pylori) infection has been linked to gastric cancer. The factors that promote carcinogenesis remain unknown. Epidermal growth factor (EGF) has been shown to be a potent epithelial mitogen and oncoprotein when sustained over expression occurs. Our aim was to compare gastric mucosal levels of EGF and its receptor (EGFR) among controls, H. pylori infected subjects, and subjects following H. pylori eradication using quantitative flow cytometric analysis. METHODS: Patients referred for evaluation of dyspepsia underwent EGD and six antral biopsies were performed (two each for rapid urease testing (RUT), histopathology, and flow cytometry). Controls were those found to be H. pylori negative while subjects had confirmed infection. The study patients were treated, then had repeat EGD with biopsies. RESULTS: There were 17 controls and 28 cases. Mean EGF and EGFR values were 2.69 and 2.46 for controls and 4.67 and 4.64 for subjects. Subjects' mean EGF was 73% higher ($p = .035$) and EGFR was 88% higher ($p = 0.029$) than controls. After treatment, the subjects' mean values declined 55% ($p = 0.0001$) for EGF and 40% ($p = 0.002$) for EGFR. Three subjects had persistent infection and showed no change in their EGF/EGFR levels. No difference was found among factor levels with respect to endoscopic findings. CONCLUSIONS: Both EGF and EGFR from gastric antral biopsies are increased nearly 2-fold in infection with H. pylori. Infection eradication reduces levels of both factors to those of controls. One major pathogenic mechanism for gastric mucosal hyperproliferation and possibly carcinogenesis related to H. pylori may be the over expression of EGF and increased receptor density of EGFR on gastric mucosal cells.

Tags: Female; Male

Descriptors: *Epidermal Growth Factor--metabolism--ME; *Gastric Mucosa --metabolism--ME; *Helicobacter Infections--drug therapy--DT; *Helicobacter pylori; *Receptor, Epidermal Growth Factor--metabolism--ME; Adult; Antacids --therapeutic use--TU; Anti-Ulcer Agents--therapeutic use--TU; Antibiotics, Combined--therapeutic use--TU; Biopsy; Bismuth--therapeutic use--TU; Chronic Disease; Flow Cytometry; Gastric Mucosa--pathology--PA; Gastritis --microbiology--MI; Gastritis--pathology--PA; Helicobacter Infections

--metabolism--ME; Helicobacter Infections--microbiology--MI; Helicobacter pylori--isolation and purification--IP; Humans; Middle Aged; Omeprazole --therapeutic use--TU; Organometallic Compounds--therapeutic use--TU; Prospective Studies; Salicylates--therapeutic use--TU
CAS Registry No.: 0 (Antacids); 0 (Anti-Ulcer Agents); 0 (Antibiotics, Combined); 0 (Organometallic Compounds); 0 (Salicylates); 14882-18-9 (bismuth subsalicylate); 62229-50-9 (Epidermal Growth Factor); 73590-58-6 (Omeprazole); 7440-69-9 (Bismuth)
Enzyme No.: EC 2.7.1.112 (Receptor, Epidermal Growth Factor)
Record Date Created: 19991027
Record Date Completed: 19991027

7/9/2
DIALOG(R)File 155:MEDLINE(R)

13528446 PMID: 10499475
Characteristics of gastric-vein lymphocytes with regard to the immune response to Helicobacter pylori.
Tricerri A; Guidi L; Frasca D; Costanzo M; Errani A R; Riccioni M E; Barattini P; Vangeli M; Bartoloni C; Coppola R; Doria G; Gasbarrini G
Institute of Internal and Geriatric Medicine, Universita Cattolica del Sacro Cuore, and Laboratory of Immunology, ENEA-CRE, Casaccia, Rome, Italy.
Scandinavian journal of gastroenterology (NORWAY) Aug 1999, 34 (8) p757-64, ISSN 0036-5521 Journal Code: 0060105
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
BACKGROUND: Between peripheral blood and tissue-infiltrating lymphocytes there is an intermediate compartment, the blood of the organ-draining vessels, which could show unusual features. The aim of the present study was to analyse the characteristics of the lymphocytes from the stomach-draining vessels and the cytokine secretion by these lymphocytes. The CagA-mediated lymphocyte activation in Helicobacter pylori-infected subjects and the humoral response to this antigen were evaluated and correlated with clinical data. METHODS: We studied lymphocyte proliferation either with mitogens or with the CagA antigen and cytokine production and IgG anti-CagA by means of an enzyme-linked immunosorbent assay in peripheral blood and gastric-vein blood obtained during surgical intervention. RESULTS: We showed higher proliferative response and cytokine production in lymphocytes from the gastric vein. The mitogenic response to the CagA antigen was highly specific but poorly sensitive for the H. pylori infection in both the compartments. The overall cytokine profile in our patients affected by non-ulcer disease was of the Th0 type. CONCLUSIONS: Gastric-vein-derived lymphocytes seem to show unusual features, as they behave like peripheral blood lymphocytes but show higher responses to all the tested stimuli. It is possible that the interaction of the lymphocytes with the mucosal environment could activate the synthetic mechanisms, making the cells more 'responsive' to the stimulation. The CagA antigen is able to induce a specific T-lymphocyte response and is therefore a valid candidate antigen for the development of a vaccine.
Tags: Female; Male
Descriptors: *Antigens, Bacterial; *Cytokines--biosynthesis--BI; *Gastrointestinal Diseases--immunology--IM; *Gastrointestinal Diseases --microbiology--MI; *Helicobacter Infections--immunology--IM; *Helicobacter pylori--immunology--IM; *Leukocytes, Mononuclear--immunology--IM; *Lymphocy

tes--immunology--IM; *Stomach--blood supply--BS; Adult; Bacterial Proteins --immunology--IM; Cytokines--blood--BL; Flow Cytometry; Gastrointestinal Diseases--surgery--SU; Helicobacter Infections--microbiology--MI; Helicoba cter Infections--surgery--SU; Humans; Leukocytes, Mononuclear--secretion --SE; Lymphocyte Activation; Lymphocyte Subsets; Lymphocytes--secretion--SE ; Middle Aged; Statistics, Nonparametric; Stomach--immunology--IM; Stomach --microbiology--MI; Veins--immunology--IM
CAS Registry No.: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Cytokines); 0 (cagA protein, Helicobacter pylori)
Record Date Created: 19991102
Record Date Completed: 19991102

7/9/3
DIALOG(R)File 155:MEDLINE(R)

13497052 PMID: 10466876
Effect of an aluminum hydroxide-magnesium hydroxide combination drug on adhesion, IL-8 inducibility, and expression of HSP60 by Helicobacter pylori.
Kamiya S; Yamaguchi H; Osaki T; Taguchi H; Fukuda M; Kawakami H; Hirano H
Dept. of Microbiology, Kyorin University School of Medicine, Mitaka, Tokyo, Japan.
Scandinavian journal of gastroenterology (NORWAY) Jul 1999, 34 (7) p663-70, ISSN 0036-5521 Journal Code: 0060105
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
BACKGROUND: Co-magaldrox (Maalox) is used world-wide as an antacid and as a cytoprotective agent for gastritis and peptic ulcer diseases. We examined the effects of co-magaldrox on Helicobacter pylori. METHODS: Adhesion of H. pylori to human gastric epithelial cells (MKN45) was evaluated by flow cytometry. Morphologic changes in H. pylori caused by co-magaldrox were determined by scanning electron microscopy. Induction of interleukin-8 (IL-8) from MKN45 cells was examined with enzyme-linked immunosorbent assay, and the intracellular and extracellular expression of heat-shock protein 60 (HSP60) was analyzed with sodium dodecyl sulphate-polyacrylamide gel electrophoresis and flow cytometry. RESULTS: Adhesion of H. pylori to MKN 45 cells was significantly inhibited by 1.25%-5% comagaldrox. H. pylori aggregated with co-magaldrox according to an electron microscopic examination. IL-8 secretion from MKN45 cells after H. pylori infection was also inhibited by co-magaldrox. Extracellular expression of HSP60 on the surface of H. pylori was decreased after treatment with comagaldrox, whereas the intracellular synthesis of HSP60 was not. HSP60-induced IL-8 secretion was significantly inhibited by co-magaldrox in a dose-dependent manner. CONCLUSIONS: These results show that co-magaldrox suppressed the expression of the following virulence factors: adhesion, IL-8 inducibility, and expression of extracellular HSP60. Therefore, co-magaldrox is a potent anti-H. pylori and cytoprotective drug.
Descriptors: *Aluminum Hydroxide--pharmacology--PD; *Antacids --pharmacology--PD; *Bacterial Adhesion--drug effects--DE; *Gastric Mucosa --microbiology--MI; *Helicobacter pylori--drug effects--DE; *Magnesium Hydroxide--pharmacology--PD; Bacterial Adhesion--physiology--PH; Cell Line; Chaperonin 60--biosynthesis--BI; Drug Combinations; Electrophoresis, Polyacrylamide Gel; Flow Cytometry; Gastric Mucosa--physiology--PH; Helicobacter pylori--physiology--PH; Helicobacter pylori--ultrastructure

--adverse effects--AE; Metronidazole--therapeutic use--TU; Organometallic Compounds--adverse effects--AE; Organometallic Compounds--therapeutic use --TU; Receptors, Interleukin-2--analysis--AN; Receptors, Interleukin-2 --drug effects--DE; T-Lymphocytes--drug effects--DE; T-Lymphocytes --immunology--IM; T-Lymphocytes--pathology--PA; Treatment Outcome
CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (Organometallic Compounds); 0 (Receptors, Interleukin-2); 207137-56-2 (Interleukin-4); 26787-78-0 (Amoxicillin); 443-48-1 (Metronidazole); 57644-54-9 (bismuth tripotassium dicitrate); 82115-62-6 (Interferon Type II)
Record Date Created: 19990922
Record Date Completed: 19990922

7/9/5
DIALOG(R)File 155:MEDLINE(R)

12693690 PMID: 10616757
Reactivity of monoclonal antibody to HSP60 homologue of Helicobacter pylori with human gastric epithelial cells and induction of IL-8 from these cells by purified H. pylori HSP60.
Yamaguchi H; Osaki T; Kurihara N; Taguchi H; Kamiya S
Department of Microbiology, Kyorin University School of Medicine, Mitaka, Tokyo, Japan.
Journal of gastroenterology (JAPAN) 1999, 34 Suppl 11 p1-5, ISSN 0944-1174 Journal Code: 9430794
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The monoclonal antibody (mAb) designated H20, which recognizes heat shock protein 60 (HSP60) of Helicobacter pylori, was previously established; and the epitope recognized by the mAb was shown to be species-specific. Using immunohistochemical staining of six gastric biopsy specimens with the H20 mAb, gastric epithelial cells of four biopsy samples stained positively. Flow cytometric analysis showed that H20 mAb reacted with primary human gastric epithelial (PHGE) cells, though the reactivities of the mAbs were different among the PHGE cells prepared. These results indicate that the species-specific epitope recognized by H20 mAb exists on human gastric cells. In addition, affinity-purified HSP60 from H. pylori by H20 mAb induced interleukin-8 (IL-8) secretion from PHGE cells (in one of four cases). These results indicate that H. pylori HSP60 induces IL-8 secretion from human gastric cells, and the levels of IL-8 differ among the various prepared PHGE cells.
Descriptors: *Chaperonin 60--immunology--IM; *Gastric Mucosa--immunology --IM; *Gastritis--immunology--IM; *Helicobacter Infections--immunology--IM; *Helicobacter pylori; *Interleukin-8--secretion--SE; Antibodies, Monoclonal --immunology--IM; Antibody Specificity; Epithelial Cells--immunology--IM; Epitopes--immunology--IM; Flow Cytometry; Gastric Mucosa--cytology--CY; Gastritis--microbiology--MI; Helicobacter pylori--immunology--IM; Helicobacter pylori--physiology--PH; Humans; Immunohistochemistry; Stomach Neoplasms--immunology--IM; Stomach Neoplasms--pathology--PA; Tumor Cells, Cultured
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Chaperonin 60); 0 (Epitopes); 0 (Interleukin-8)
Record Date Created: 20000201
Record Date Completed: 20000201

Type II)
Record Date Created: 19991102
Record Date Completed: 19991102

7/9/7
DIALOG(R)File 155:MEDLINE(R)

12607922 PMID: 10358446
[Effect of H. pylori eradication regimes on the proliferation index of gastric mucosa]
Vliv eradikacnich rezimuu H. pylori na proliferacni index zaludecni sliznice.
Hep A; Zaloudik J; Janakova J; Habanec B; Prasek J; Dolina J; Dite P
III. interni klinika-gastroenterologicka FN, Brno.
Vnitrni lekarstvi (CZECH REPUBLIC) Aug 1998, 44 (8) p447-50, ISSN 0042-773X Journal Code: 0413602
Publishing Model Print
Document type: Journal Article ; English Abstract
Languages: CZECH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Eradication regimes with the blocking agent of the proton pump and without it do not influence the activity of cell division after treatment of Helicobacter pylori (H.p.) when using cytoflowmetric evaluation. The non-significant difference in proliferation activity of the gastric mucosa after treatment of H.p. can be also a sign of more rapid repair of the gastric mucosa after elimination of the inflammatory elements.
Tags: Female; Male; Research Support, Non-U.S. Gov't
Descriptors: *Gastric Mucosa--pathology--PA; *Gastritis--drug therapy--DT ; *Helicobacter Infections--drug therapy--DT; *Helicobacter pylori; Cell Division; Flow Cytometry; Gastritis--microbiology--MI; Gastritis --pathology--PA; Helicobacter Infections--pathology--PA; Humans
Record Date Created: 19990625
Record Date Completed: 19990625

7/9/8
DIALOG(R)File 155:MEDLINE(R)

12555847 PMID: 9872496
A virulence factor of Helicobacter pylori: role of heat shock protein in mucosal inflammation after H. pylori infection.
Kamiya S; Yamaguchi H; Osaki T; Taguchi H
Department of Microbiology, Kyorin University School of Medicine, Tokyo, Japan.
Journal of clinical gastroenterology (UNITED STATES) 1998, 27 Suppl 1 pS35-9, ISSN 0192-0790 Journal Code: 7910017
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Among the various virulence factors of Helicobacter pylori the role of its heat shock protein 60 (HSP60, HspB) in mucosal inflammation after H. pylori infection was examined. In flow cytometric analysis, the expression

of HSP60 on the cell surface was different, depending on the H. pylori strain used. The HSP60 epitope was also detected on the surface of both human gastric cancer cells (MKN45, KATOIII, and MKN28) and human gastric biopsy specimens. The intensity of the expression of HSP60 on the cell surface correlated significantly with the adhesion of H. pylori to MKN45 cells, but not with urease activity and production of vacuolating cytotoxin. A monoclonal antibody to H. pylori HSP60 inhibited the adhesion of H. pylori to MKN45 cells. These results suggest that HSP60 of H. pylori might act as an important virulence factor after H. pylori infection.

Descriptors: *Chaperonin 60--metabolism--ME; *Gastric Mucosa --microbiology--MI; *Gastritis--physiopathology--PP; *Helicobacter Infections--physiopathology--PP; *Helicobacter pylori--pathogenicity--PY; Antibodies, Monoclonal--immunology--IM; Bacterial Adhesion--immunology--IM; Biopsy; Chaperonin 60--immunology--IM; Flow Cytometry; Gastric Mucosa --pathology--PA; Gastritis--microbiology--MI; Gastritis--pathology--PA; Helicobacter Infections--microbiology--MI; Helicobacter Infections --pathology--PA; Helicobacter pylori--isolation and purification--IP; Humans; Immunohistochemistry; Stomach Neoplasms--microbiology--MI; Stomach Neoplasms--pathology--PA; Stomach Neoplasms--physiopathology--PP; Tumor Cells, Cultured; Virulence

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Chaperonin 60)

Record Date Created: 19990318

Record Date Completed: 19990318

7/9/9

DIALOG(R)File 155:MEDLINE(R)

12419345 PMID: 9731889

Increased cell proliferation of the gastric mucosa in first-degree relatives of gastric carcinoma patients.

Meining A; Hackelsberger A; Daenecke C; Stolte M; Bayerdorffer E; Ochsenkuhn T

Department of Internal Medicine II, Klinikum Grosshadern, University of Munich, Germany.

Cancer (UNITED STATES) Sep 1 1998, 83 (5) p876-81, ISSN 0008-543X Journal Code: 0374236

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

BACKGROUND: Studies not considering Helicobacter pylori infection have suggested the presence of a hereditary risk for gastric carcinoma. However, other studies have identified intrafamilial clustering of H. pylori infection as a causal factor in gastric carcinogenesis. This prompted the authors to study the effect of H. pylori and hereditary factors on the proliferation of gastric mucosa because hyperproliferation appears to be an early step in carcinogenesis. METHODS: In a total of 39 patients (19 first-degree relatives of patients with gastric carcinoma and 20 dyspeptic controls), 2 biopsy specimens each from the antrum and corpus were examined histologically. In addition, crude nuclei fractions were prepared from other biopsy specimens obtained in the same manner. Nuclei were fixed in 70% ethanol and stained with propidium iodine prior to measurement. A cell cycle analysis was performed using a flow cytometer. For analysis a proliferative index (PI) (percentage of nuclei in the S- and G2/M-phases) was calculated. RESULTS: In comparison with control patients, first-degree relatives of gastric carcinoma patients had increased mucosal proliferation

of the antrum (Student's t test, P = 0.017). After excluding patients with H. pylori infection (12 in each group), relatives of gastric carcinoma patients had significantly increased proliferation not only in the antrum (PI: 16.5 vs. 12.1; P = 0.043), but also in the corpus (PI: 17.2 vs. 13.0; P = 0.024). CONCLUSIONS: A family history of gastric carcinoma may increase the risk for developing gastric carcinoma via mucosal hyperproliferation, irrespective of H. pylori infection.

Tags: Female; Male

Descriptors: *Gastric Mucosa--pathology--PA; *Stomach Neoplasms--genetics --GE; Cell Division; Flow Cytometry; Helicobacter Infections--pathology--PA ; Helicobacter pylori; Humans; Middle Aged; Pyloric Antrum--pathology--PA; Risk Factors

Record Date Created: 19980918

Record Date Completed: 19980918

7/9/10

DIALOG(R)File 155:MEDLINE(R)

12358623 PMID: 9671122

A novel flow cytometric assay for quantitating adherence of Helicobacter pylori to gastric epithelial cells.

Logan R P; Robins A; Turner G A; Cockayne A; Borriello S P; Hawkey C J

Division of Gastroenterology, University Hospital, Nottingham, UK. robert.logan@nottingham.ac.uk

Journal of immunological methods (NETHERLANDS) Apr 1 1998, 213 (1) p19-30, ISSN 0022-1759 Journal Code: 1305440

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Adherence may be an important virulence factor for Helicobacter pylori. Current methods available for quantitation of adherence are time consuming and liable to observer error. A new direct technique for fluorescent labelling of bacteria has been developed to quantitate adherence of H. pylori to epithelial cells by fluorescence activated cell sorting (FACS). Type strains of H. pylori, H. mustelae, H. cinaedi and H. fennelliae were grown microaerobically in broth culture for 24 h and fluorescently labelled by incubation with carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) at 37 degrees C. After washing to remove excess CFDA-SE, bacteria were co-incubated (ratio 10:1) with gastric epithelial cells at 37 degrees C for up to 24 h. After washing to remove non-adherent bacteria, epithelial cells were detached with EDTA (2 mM) and fixed with formaldehyde for flow cytometry. Adherence was quantitated both in terms of the proportion of cells with adherent H. pylori and as the mean number of adherent bacteria per cell. All H. pylori strains adhered to gastric-type epithelial cells. The proportion of cells with bound bacteria varied from 40-99% and the number of bacteria per cell from 1-50, both of which correlated with microscopy (r = 0.6, and r = 0.8 respectively, n = 35). Time course studies demonstrated saturation of binding by H. pylori within 90 min. For H. mustelae, H. cinaedi and H. fennelliae the proportion of cells with bound bacteria varied from 5-15% and the mean number of bacteria per cell was < 4. Binding of H. pylori to epithelial cells could be partly blocked by pre-incubation with polyclonal anti-sera or using oligosaccharides against potential binding epitopes of gastric mucus. Fluorescent labelling of H. pylori with CFDA-SE in combination with flow cytometry provides a quick, specific, and sensitive method to quantitate in vitro the adherence of H.

pylori.
Tags: Research Support, Non-U.S. Gov't
Descriptors: *Bacterial Adhesion; *Flow Cytometry--methods--MT; *Helicobacter pylori--metabolism--ME; *Stomach--microbiology--MI; Antibodies, Bacterial--immunology--IM; Cell Line; Epithelial Cells--microbiology--MI; Fluoresceins; Fluorescent Dyes; Helicobacter pylori--immunology--IM; Humans; Reproducibility of Results; Stomach--cytology--CY; Succinimides
CAS Registry No.: 0 (5-(6)-carboxyfluorescein diacetate succinimidyl ester); 0 (Antibodies, Bacterial); 0 (Fluoresceins); 0 (Fluorescent Dyes); 0 (Succinimides)
Record Date Created: 19980805
Record Date Completed: 19980805

7/9/11
DIALOG(R)File 155:MEDLINE(R)

12265176 PMID: 9576451
An increased number of follicles containing activated CD69+ helper T cells and proliferating CD71+ B cells are found in H. pylori-infected gastric mucosa.
Terres A M; Pajares J M
Gastroenterology Service, Hospital de la Princesa and Universidad Autonoma de Madrid, Spain.
American journal of gastroenterology (UNITED STATES) Apr 1998, 93 (4) p579-83, ISSN 0002-9270 Journal Code: 0421030
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
OBJECTIVE: An immune response occurring in Helicobacter pylori-infected gastric mucosa could have a direct implication for associated pathologies. In the present study we analyzed the expression of the immune activation, proliferation, and phenotype markers by immune cell subpopulations in H. pylori-infected and uninfected gastric samples. METHODS: Antral gastric biopsies from both H. pylori-positive and -negative patients were processed by immunohistochemistry; gastric epithelial cells were isolated from biopsy tissue and analyzed by flow cytometry. RESULTS: Ten of the 13 biopsies that contained follicles were H. pylori positive. Follicular CD69 was expressed mainly by CD4+ T cells and the central core of follicles showed double immunopositivity for the B-cell marker CD19 and transferrin receptor. Also detected was an increase in the percentage of epithelial cells from H. pylori-positive samples expressing HLA-DR and beta2 microglobulin, compared to negative samples (61 +/- 15% vs 9 +/- 9%, p = 0.003 and 93 +/- 7% vs 52 +/- 18%, p = 0.002, respectively), whereas no variation on class I HLA was detected. CONCLUSIONS: These results suggest that chronic H. pylori infection could facilitate the persistence of follicles on which continuous follicular helper T-cell activation could lead to uncontrolled follicular B-cell proliferation. Furthermore, beta2 microglobulin expression by epithelial cells in a nonparallel way to class I HLA may indicate the possibility of nonclassical class I MHC expression on the basal surface of the epithelium.
Tags: Female; Male; Research Support, Non-U.S. Gov't
Descriptors: *Antigens, CD--analysis--AN; *Antigens, Differentiation, B-Lymphocyte--analysis--AN; *Antigens, Differentiation, T-Lymphocyte--analysis--AN; *B-Lymphocytes--immunology--IM; *Gastric Mucosa--immunology--IM; *Helicobacter Infections--immunology--IM; *Helicobacter pylori;

*Receptors, Transferrin--analysis--AN; *T-Lymphocytes, Helper-Inducer --immunology--IM; Adult; Aged; B-Lymphocytes--pathology--PA; Biopsy; CD4-Positive T-Lymphocytes--immunology--IM; CD4-Positive T-Lymphocytes --pathology--PA; Epithelial Cells--immunology--IM; Epithelial Cells --pathology--PA; Flow Cytometry; Gastric Mucosa--pathology--PA; HLA-DR Antigens--analysis--AN; Helicobacter Infections--pathology--PA; Humans; Immunohistochemistry; Lymphocyte Activation; Middle Aged; Pyloric Antrum --immunology--IM; Pyloric Antrum--pathology--PA; T-Lymphocytes, Helper-Inducer--pathology--PA; beta 2-Microglobulin--analysis--AN

CAS Registry No.: 0 (Antigens, CD); 0 (Antigens, Differentiation, B-Lymphocyte); 0 (Antigens, Differentiation, T-Lymphocyte); 0 (CD69 antigen); 0 (HLA-DR Antigens); 0 (Receptors, Transferrin); 0 (TFRC protein, human); 0 (beta 2-Microglobulin)

Record Date Created: 19980515

Record Date Completed: 19980515

7/9/12

DIALOG(R)File 155:MEDLINE(R)

12191979 PMID: 9498470

Highly significant role of Helicobacter pylori urease in phagocytosis and production of oxygen metabolites by human granulocytes.

Makristathis A; Rokita E; Labigne A; Willinger B; Rotter M L; Hirschl A M

Department of Clinical Microbiology, Hygiene Institute, University of Vienna, Austria. Athanasios.Makristathis@akh-wien.ac.at

Journal of infectious diseases (UNITED STATES) Mar 1998, 177 (3) p803-6, ISSN 0022-1899 Journal Code: 0413675

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

The contribution of Helicobacter pylori urease, the vacuolating cytotoxin VacA, and the 128-kDa protein CagA to the stimulation of human granulocytes in terms of phagocytosis and oxidative burst was evaluated. Blood was incubated with H. pylori strains and corresponding isogenic mutants lacking either the large urease subunit (UreB) or an accessory urease protein (UreG) or VacA or CagA. Phagocytosis and oxidative burst were monitored by flow cytometry. The UreB-lacking mutant was phagocytosed more efficiently ($P < .001$) and induced significantly less oxidative burst ($P < .001$) than its parental strain or the UreG-lacking mutant, which produces an enzymatically inactive urease. Values of the other mutants did not differ greatly from those of their parental strain. These data indicate inflammatory effects of H. pylori urease causing inhibition of phagocytosis and stimulation of oxidative burst by a pathway being largely independent of ammonia production.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Antigens, Bacterial; *Granulocytes--immunology--IM; *Helicobacter pylori--immunology--IM; *Phagocytosis; *Respiratory Burst; *Urease--immunology--IM; Bacterial Proteins--immunology--IM; Bacterial Toxins--immunology--IM; Flow Cytometry; Helicobacter pylori--enzymology--EN ; Humans

CAS Registry No.: 0 (Antigens, Bacterial); 0 (Bacterial Proteins); 0 (Bacterial Toxins); 0 (VacA protein, Helicobacter pylori); 0 (cagA protein, Helicobacter pylori)

Enzyme No.: EC 3.5.1.5 (Urease)

Record Date Created: 19980326

Record Date Completed: 19980326

7/9/13
DIALOG(R)File 155:MEDLINE(R)

12185082 PMID: 9492175
Growth inhibition of Helicobacter pylori by monoclonal antibody to heat-shock protein 60.
Yamaguchi H; Osaki T; Taguchi H; Hanawa T; Yamamoto T; Fukuda M; Kawakami H; Hirano H; Kamiya S
Department of Microbiology, Kyorin University School of Medicine, Mitaka, Tokyo, Japan.
Microbiology and immunology (JAPAN) 1997, 41 (12) p909-16, ISSN 0385-5600 Journal Code: 7703966
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The H20mAb recognizing the 60-kilodalton protein, which existed in the outer membrane and was induced by heat shock at 42 C, was established. The molecule recognized with the mAb was a heat-shock protein 60 (HSP60) of Helicobacter pylori. To understand the role of HSP60 on the cell surface of H. pylori, whether or not H20mAb affects the growth of H. pylori was investigated. When bacteria were cultured with H20mAb, growth was markedly inhibited after 24 hr, although an initial 5 hr-incubation with the mAb induced no significant inhibition of H. pylori growth. The 24- and 48 hr growth of the bacteria after washing to remove the mAb at 5 hr was also inhibited though the inhibitory effect was not strong. In electron microscopical analysis, the spots with high electron density in the cytoplasm of the bacteria treated with H20mAb were increased, depending on the length of incubation time from 5 to 24 hr. After 24 hr treatment with H20mAb, bacterial destruction was also observed, indicating bactericidal activity by H20mAb. These results suggest that the HSP60 on the cell surface of H. pylori might have an essential role in the growth of the bacteria.
Tags: Research Support, Non-U.S. Gov't
Descriptors: *Antibodies, Monoclonal--immunology--IM; *Chaperonin 60 --physiology--PH; *Helicobacter pylori--growth and development--GD; Amino Acid Sequence; Antibodies, Bacterial--immunology--IM; Bacterial Outer Membrane Proteins--immunology--IM; Bacterial Outer Membrane Proteins --physiology--PH; Blotting, Western; Chaperonin 60--immunology--IM; Electrophoresis, Gel, Two-Dimensional; Electrophoresis, Polyacrylamide Gel; Epitope Mapping; Flow Cytometry; Helicobacter pylori--cytology--CY; Helicobacter pylori--immunology--IM; Microscopy, Electron; Molecular Sequence Data
CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antibodies, Monoclonal); 0 (Bacterial Outer Membrane Proteins); 0 (Chaperonin 60)
Record Date Created: 19980402
Record Date Completed: 19980402

7/9/14
DIALOG(R)File 155:MEDLINE(R)

12062525 PMID: 9358084

Helicobacter pylori induces proinflammatory cytokines and major histocompatibility complex class II antigen in mouse gastric epithelial cells.

Maekawa T; Kinoshita Y; Matsushima Y; Okada A; Fukui H; Waki S; Kishi K; Kawanami C; Nakata H; Hassan S; Wakatsuki Y; Ota H; Amano K; Nakao M; Chiba T

Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, Japan.

Journal of laboratory and clinical medicine (UNITED STATES) Oct 1997, 130 (4) p442-9, ISSN 0022-2143 Journal Code: 0375375

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

Although Helicobacter pylori has been reported to stimulate the release of various cytokines from gastric tissue, it remains unknown whether normal and nontumorous gastric epithelial cells produce these cytokines. Therefore, in this study, we used a normal mouse gastric surface mucous cell line (GSM06) to determine whether gastric epithelial cells produce proinflammatory cytokines in response to H. pylori. The expression of MHC class II antigen was also examined, to investigate whether gastric epithelial cells participate in the immune response to H. pylori. In the study, GSM06 cells were incubated with H. pylori or its lipopolysaccharide (LPS). Proinflammatory cytokines were detected by Northern and Western blot analysis. The expression of MHC class II antigen was examined by fluorescence activated cell sorter (FACS) analysis. Genetic expression of proinflammatory cytokines such as interleukin-1alpha, tumor necrosis factor-alpha, and cytokine-induced neutrophil chemoattractant-2beta was enhanced by both intact and sonicated H. pylori, but not by H. pylori LPS. The expression of MHC class II antigen was induced by H. pylori more strongly than by interferon-gamma. We conclude that H. pylori induces the expression of proinflammatory cytokines and MHC class II antigen in gastric epithelial cells. Gastric epithelial cells may act as antigen-presenting cells and participate in the immune response to H. pylori infection.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Chemokines, CXC; *Cytokines--biosynthesis--BI; *Gastric Mucosa--immunology--IM; *Gastric Mucosa--microbiology--MI; *Helicobacter pylori--physiology--PH; *Histocompatibility Antigens Class II--biosynthesis --BI; *Intercellular Signaling Peptides and Proteins; Animals; Cell Line; Chemotactic Factors--biosynthesis--BI; Chemotactic Factors--genetics--GE; Cytokines--genetics--GE; Escherichia coli; Flow Cytometry; Gene Expression; Growth Substances--biosynthesis--BI; Growth Substances--genetics--GE; Helicobacter pylori--immunology--IM; Histocompatibility Antigens Class II --genetics--GE; Histocompatibility Antigens Class II--immunology--IM; Histocytochemistry; Inflammation; Interferon Type II--pharmacology--PD; Interleukin-1--biosynthesis--BI; Interleukin-1--genetics--GE; Kinetics; Lipopolysaccharides--pharmacology--PD; Mice; Polymyxin B--pharmacology--PD; RNA, Messenger--genetics--GE; RNA, Messenger--metabolism--ME; Tumor Necrosis Factor-alpha--biosynthesis--BI; Tumor Necrosis Factor-alpha --genetics--GE

CAS Registry No.: 0 (Chemokines, CXC); 0 (Chemotactic Factors); 0 (Cxcl1 protein, mouse); 0 (Cytokines); 0 (Growth Substances); 0 (Histocompatibility Antigens Class II); 0 (Intercellular Signaling Peptides and Proteins); 0 (Interleukin-1); 0 (Lipopolysaccharides); 0 (RNA, Messenger); 0 (Tumor Necrosis Factor-alpha); 1404-26-8 (Polymyxin B); 82115-62-6 (Interferon Type II)

Record Date Created: 19971125

Record Date Completed: 19971125

7/9/15
DIALOG(R)File 155:MEDLINE(R)

12030495 PMID: 9322506
Gastritis in urease-immunized mice after Helicobacter felis challenge may be due to residual bacteria.
Ermak T H; Ding R; Ekstein B; Hill J; Myers G A; Lee C K; Pappo J; Kleanthous H K; Monath T P
OraVax, Inc., Cambridge, Massachusetts, USA.
Gastroenterology (UNITED STATES) Oct 1997, 113 (4) p1118-28, ISSN 0016-5085 Journal Code: 0374630
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS
BACKGROUND & AIMS: Oral immunization with recombinant Helicobacter pylori urease (rUre) coadministered with a mucosal adjuvant protects mice against challenge with Helicobacter felis. In this study, the duration of protection and gastritis after challenge were characterized at sequential time intervals up to 1 year. METHODS: Outbred Swiss-Webster mice were orally immunized with rUre plus adjuvant and examined for the presence of H. felis infection and leukocyte infiltration into the gastric mucosa. RESULTS: When defined by gastric urease activity, 70%-95% of rUre-immunized mice were protected for between 2 and 57 weeks. Challenge with H. felis increased the inflammatory response in the gastric mucosa of rUre-immunized mice, which also had elevated CD4+ and CD8+ T cells. The CD8+ cells represented a population of gastric intraepithelial cells, which expressed the mucosal alpha E-integrin. Epithelial changes consisting of parietal cell loss and hyperplasia of the epithelium occurred in approximately 20% of the mice. Antimicrobial triple therapy significantly decreased the degree of gastritis and epithelial alteration in the stomach. CONCLUSIONS: These results indicate that oral immunization of mice with rUre produces a long-lasting inhibition of H. felis infection but that residual bacteria may produce a persistent lymphocytic infiltration under these experimental conditions.
Tags: Female
Descriptors: *Bacterial Vaccines; *Gastric Mucosa--immunology--IM; *Gastritis--microbiology--MI; *Helicobacter--isolation and purification--IP; *Helicobacter Infections--immunology--IM; *Helicobacter pylori --immunology--IM; *Urease--immunology--IM; *Vaccines, Synthetic; Animals; Flow Cytometry; Gastric Mucosa--microbiology--MI; Gastric Mucosa --pathology--PA; Helicobacter--enzymology--EN; Helicobacter Infections --complications--CO; Helicobacter pylori--enzymology--EN; Immunophenotyping; Integrins--biosynthesis--BI; Intestinal Mucosa--immunology--IM; Intestinal Mucosa--microbiology--MI; Intestinal Mucosa--pathology--PA; Metaplasia; Mice; Receptors, Antigen, T-Cell, alpha-beta--biosynthesis--BI; T-Lymphocyte Subsets--immunology--IM; T-Lymphocyte Subsets--pathology--PA; Urease--analysis--AN
CAS Registry No.: 0 (Bacterial Vaccines); 0 (Integrins); 0 (Receptors, Antigen, T-Cell, alpha-beta); 0 (Vaccines, Synthetic)
Enzyme No.: EC 3.5.1.5 (Urease)
Record Date Created: 19971023
Record Date Completed: 19971023

7/9/16
DIALOG(R)File 155:MEDLINE(R)

11941111 PMID: 9222708
Cellular proliferation and ploidy of the gastric mucosa: the role of Helicobacter pylori.
Abdel-Wahab M; Attallah A M; Elshal M F; Abdel-Raouf M; Zalata K R; el-Ghawalby N; Ezzat F
Biotechnology Research Laboratories, Mansoura University, Egypt.
Hepato-gastroenterology (GREECE) May-Jun 1997, 44 (15) p880-5, ISSN 0172-6390 Journal Code: 8007849
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
BACKGROUND/AIMS: Recently, H. pylori has been recognized as a risk factor for gastric adenocarcinoma. As such, we have analyzed the DNA content of gastric epithelial cells in an attempt to reveal the role of H. pylori in gastric carcinogenesis. METHODOLOGY: Fifty-three subjects presented with gastric dyspepsia, 39 males and 14 females, with a mean age of 42.15 (+/- 13.16) years. They were referred to the out-patient clinic to undergo endoscopic examination for the first time. Biopsy specimens from the antrum of each subject were subjected to culture for the presence of H. pylori histologic diagnosis, and DNA flow cytometry for the analysis of cellular proliferation and DNA policy. RESULTS: The endoscopic diagnoses were normal appearance (12), Gastric ulcer (12), duodenal ulcer (29). Thirty-eight (72%) subjects were positive, and 15 (28%) subjects were negative for H. pylori. Abnormal DNA-content (aneuploidy) was found in specimens from the antrums of 3 patients, 2 patients with duodenal ulcers (DU, and one with a gastric ulcer (GU). The cellular proliferation detected by flow cytometry in the form of proliferative index (PI; percentage of cells in the DNA S and G2M phases) was 27.88 (+/- 12.48) and 14.17 (+/-2.94) in the antrums of those positive and negative for H. pylori, respectively. A very significant increase in the PI ($p < 0.005$) was found between subjects positive and negative for H. pylori. Patients with DU and H pylori infection had the highest PI, and the PI was significantly higher than in patients with DU, but without infection. Regarding histology, there was a significant increase in the PI in the presence of H. pylori infection in either CAG or dysplasia groups as compared to cases without infection in the same groups. CONCLUSION: These results show that H. pylori infection is associated with changes in the DNA-content and cellular proliferative activity, suggesting that H. pylori may be implicated in gastric carcinogenesis. Also, the significant increase in the PI along the progression of severity of the disease suggests that measuring this parameter might allow more accurate monitoring of patients, so that a targeted therapeutic protocol may be defined.
Tags: Female; Male
Descriptors: *Gastric Mucosa--pathology--PA; *Gastritis--pathology--PA; *Helicobacter Infections--pathology--PA; *Helicobacter pylori; Adult; Cell Division; DNA--genetics--GE; Flow Cytometry; Gastric Mucosa--metabolism--ME ; Gastritis--complications--CO; Gastritis--microbiology--MI; Helicobacter Infections--complications--CO; Humans; Middle Aged; Peptic Ulcer --microbiology--MI; Peptic Ulcer--pathology--PA; Ploidies; Stomach Neoplasms--microbiology--MI
CAS Registry No.: 9007-49-2 (DNA)
Record Date Created: 19970911
Record Date Completed: 19970911

7/9/17
DIALOG(R)File 155:MEDLINE(R)

11927333 PMID: 9207264
Absence of effect of Lewis A and Lewis B expression on adherence of Helicobacter pylori to human gastric cells.
Clyne M; Drumm B
Department of Paediatrics, University College Dublin, The Childrens Research Centre, Our Lady's Hospital for Sick Children, Crumlin, Ireland. marg@crumlin.ucd.ie
Gastroenterology (UNITED STATES) Jul 1997, 113 (1) p72-80, ISSN 0016-5085 Journal Code: 0374630
Publishing Model Print; Comment in Gastroenterology. 1998 Mar;114(3) 621; Comment in PMID 9496961
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS
BACKGROUND & AIMS: Lewis b blood group antigen and antibodies to Lewis b inhibit the binding of stationary-phase Helicobacter pylori organisms to fixed sections of gastric tissue. The aim of this study was to determine the effect of expression of Lewis a and Lewis b on binding of H. pylori to primary gastric cells. METHODS: ABO and Lewis blood types were determined for 13 individuals. Cells were isolated from gastric biopsy specimens by collagenase digestion. Lewis antigen expression and adherence of H. pylori to the cells were quantitated using flow cytometry. RESULTS: Two of the three nonsecretors were found to express Lewis b on their cells. Nineteen of 19 individuals expressed Lewis a on their cells and 18 of 19 expressed Lewis b. The percentage of cells expressing Lewis antigens varied from individual to individual. H. pylori binding was independent of expression of Lewis antigens. Preincubation of cells with antibodies to Lewis antigens did not inhibit the adherence. CONCLUSIONS: H. pylori adheres to isolated human gastric cells in a manner that is not dependent on Lewis antigen expression on these cells, and expression of Lewis antigens on the surface of gastric cells is not dependent on Lewis antigen expression on erythrocytes.
Tags: Research Support, Non-U.S. Gov't
Descriptors: *Bacterial Adhesion--physiology--PH; *Gastric Mucosa --metabolism--ME; *Helicobacter pylori--metabolism--ME; *Lewis Blood-Group System--immunology--IM; Cells, Cultured; Child; Erythrocytes; Flow Cytometry; Gastric Mucosa--cytology--CY; Gastritis--microbiology--MI; Helicobacter Infections--blood--BL; Helicobacter Infections--microbiology --MI; Helicobacter Infections--pathology--PA; Humans; Peptic Ulcer --microbiology--MI; Tumor Cells, Cultured
CAS Registry No.: 0 (Lewis Blood-Group System)
Record Date Created: 19970723
Record Date Completed: 19970723

7/9/18
DIALOG(R)File 155:MEDLINE(R)

11807531 PMID: 9060870
Studies on the relationship between adhesive activity and haemagglutination by Helicobacter pylori.

Osaki T; Yamaguchi H; Taguchi H; Kumada J; Ogata S; Kamiya S
Division of Flow Cytometry, Kyorin University School of Medicine, Tokyo, Japan.
Journal of medical microbiology (ENGLAND) Feb 1997, 46 (2) p117-21, ISSN 0022-2615 Journal Code: 0224131
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The adhesion of Helicobacter pylori to gastric carcinoma cells (MKN45, KatoIII and MKN28) and Intestine-407 cells was tested by flow cytometric analysis. The mean adhesion rates of H. pylori strains to MKN45, KatoIII and Intestine-407 cells were 90.5, 42.7 and 15.1%, respectively. There was no statistical correlation between the adhesion rates to MKN45 cells and haemagglutination (HA) activity of H. pylori strains, although H. pylori strains with high HA activity with human type O erythrocytes tended to adhere effectively to MKN45 cells. No correlation between adhesion and production of vacuolating toxin was observed.
Descriptors: *Bacterial Adhesion; *Carcinoma--microbiology--MI; *Helicobacter pylori--metabolism--ME; *Hemagglutination; *Stomach Neoplasms --microbiology--MI; Animals; Bacterial Proteins--biosynthesis--BI; Bacterial Toxins--biosynthesis--BI; Carcinoma--pathology--PA; Cattle; Chickens; Flow Cytometry; Guinea Pigs; Helicobacter pylori--pathogenicity --PY; Hemagglutination Tests; Horses; Humans; Rabbits; Sheep; Stomach Neoplasms--pathology--PA; Tumor Cells, Cultured
CAS Registry No.: 0 (Bacterial Proteins); 0 (Bacterial Toxins); 0 (VacA protein, Helicobacter pylori)
Record Date Created: 19970324
Record Date Completed: 19970324

7/9/19
DIALOG(R)File 155:MEDLINE(R)
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11661924 PMID: 8975986
Il-2 receptor expression on gastric mucosa T lymphocytes is enhanced in duodenal ulcer patients compared with non-ulcer dyspeptic patients.
Ihan A; Tepez B; Kavcic I; Gubina M
Institute of Microbiology, Medical Faculty, Ljubljana, Slovenia.
Hepato-gastroenterology (GREECE) Nov-Dec 1996, 43 (12) p1665-70, ISSN 0172-6390 Journal Code: 8007849
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
BACKGROUND/AIMS: Helicobacter pylori infects an estimated 50% of the world population, however only a small proportion of individuals develop clinical symptoms of gastritis, peptic ulceration or gastric cancer. The variations in disease presentation may be due to differences in bacterial virulence and/or immune response to the pathogen. This study examined the expression of IL-2 receptor and ICAM-1 molecules on gastric mucosa infiltrating T lymphocytes in two groups of H. pylori infected patients: one group with an active ulcer disease and the other with non-ulcerative chronic gastritis. MATERIAL AND METHODS: T lymphocytes were isolated from gastric mucosa biopsies by using mechanical and enzymatic tissue

desegregation. Ficoll-purified lymphocytes were incubated with monoclonal antibodies and analyzed by using 3-color flow cytometry analysis for the IL-2 receptor (CD25) and ICAM-1 molecule (CD54) expression. Lymphocytes from 37 Helicobacter pylori infected patients with severe gastric mucosa infiltration (G2 and G3 histological type in Sydney classification) were analyzed; 18 patients had at least 5-year history of duodenal ulcer disease (group A) and 19 patients had at least 3-year history of non-ulcer dyspeptic disease (group B). RESULTS: We demonstrated a significant increase in IL-2 receptor expression on gastric mucosa T cells in ulcer patients (group A) compared with non-ulcer dyspeptic patients (group B). However, no difference in CD54 expression was found between the two groups of patients. CONCLUSIONS: Our results suggest the importance of the local immune response in the development of H. pylori related diseases. Also some interesting points for further study of the association between immune response against H. pylori and the development of duodenal ulcer disease were indicated.

Tags: Female; Male

Descriptors: *Duodenal Ulcer--metabolism--ME; *Gastric Mucosa--metabolism --ME; *Gastritis--metabolism--ME; *Helicobacter Infections--metabolism--ME; *Receptors, Interleukin-2--metabolism--ME; *T-Lymphocytes--metabolism--ME; Adult; Aged; Duodenal Ulcer--immunology--IM; Duodenal Ulcer--microbiology --MI; Duodenal Ulcer--pathology--PA; Flow Cytometry; Gastric Mucosa --cytology--CY; Gastric Mucosa--immunology--IM; Gastric Mucosa--pathology --PA; Gastritis--immunology--IM; Gastritis--microbiology--MI; Gastritis --pathology--PA; Helicobacter Infections--immunology--IM; Helicobacter pylori; Humans; Immunophenotyping; Intercellular Adhesion Molecule-1 --metabolism--ME; Middle Aged

CAS Registry No.: 0 (Receptors, Interleukin-2); 126547-89-5 (Intercellular Adhesion Molecule-1)

Record Date Created: 19970331

Record Date Completed: 19970331

7/9/20

DIALOG(R)File 155:MEDLINE(R)

11651123 PMID: 8959511

Induction and epitope analysis of Helicobacter pylori heat shock protein.

Yamaguchi H; Osaki T; Taguchi H; Hanawa T; Yamamoto T; Kamiya S

Department of Microbiology, Kyorin University School of Medicine, Tokyo, Japan.

Journal of gastroenterology (JAPAN) Nov 1996, 31 Suppl 9 p12-5, ISSN 0944-1174 Journal Code: 9430794

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Induction of heat shock proteins (HSPs) was analyzed in Helicobacter pylori strains. With heat shock at 42 degrees C, a synthesized 60 kDa-HSP (HSP60) was detected on autoradiography. The expression of HSP60 on the cell surface of H. pylori was examined by flow cytometric analysis. All strains used in this study expressed HSP60 on the cell surface, although the intensity differed among the strains, depending on culture conditions. The reactivity of a monoclonal antibody (mAb), 3C8, directed against bacterial HSP60, with HSP60 derived from ten strains of H. pylori and with human gastric carcinoma cell HSP60 was examined by immunoblot analysis. An epitope that reacted with the mAb was detected in the HSP60 of H. pylori

and on the surface of human gastric carcinoma cells.
Descriptors: *Chaperonin 60--biosynthesis--BI; *Epitopes--analysis--AN; *Helicobacter pylori--metabolism--ME; Antibodies, Monoclonal; Chaperonin 60 --immunology--IM; Flow Cytometry; Helicobacter pylori--immunology--IM; Humans; Immunoblotting; Stomach Neoplasms--immunology--IM; Stomach Neoplasms--metabolism--ME; Tumor Cells, Cultured
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Chaperonin 60); 0 (Epitopes)
Record Date Created: 19970410
Record Date Completed: 19970410

7/9/21
DIALOG(R)File 155:MEDLINE(R)

11536657 PMID: 8849701
Flow cytometric analysis of the heat shock protein 60 expressed on the cell surface of Helicobacter pylori.
Yamaguchi H; Osaki T; Taguchi H; Hanawa T; Yamamoto T; Kamiya S
Department of Microbiology, Kyorin University School of Medicine, Mitaka, Tokyo, Japan.
Journal of medical microbiology (ENGLAND) Oct 1996, 45 (4) p270-7, ISSN 0022-2615 Journal Code: 0224131
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The expression of a 60-kDa heat shock protein (HSP60) on the cell surface of Helicobacter pylori was analysed by flow cytometry with polyclonal antibody directed to HSP60. All 13 strains of H. pylori examined expressed HSP60 on the cell surface, although the intensity of expression was different among the strains and depended on culture conditions. There was a correlation between the intensity of HSP60 expressed on the cell surface and the rate of adherence to human gastric carcinoma cells (MKN45) by H. pylori, but not with urease activity and production of vacuolating toxin. By flow cytometric analysis with monoclonal antibody (MAb) 3C8 against HSP60, the reactive epitope in the HSP60 of H. pylori was detected on the surface of MKN45 cells. Furthermore, it was shown that gastric epithelial cells were positively stained with MAb 3C8 in one of two biopsy specimens examined. These results suggest that there is a common epitope showing homology between H. pylori HSP60 and human gastric epithelial cells.
Descriptors: *Chaperonin 60--analysis--AN; *Helicobacter pylori --metabolism--ME; Animals; Antibodies, Bacterial--immunology--IM; Antibodies, Monoclonal--immunology--IM; Bacterial Adhesion; Bacterial Proteins--biosynthesis--BI; Bacterial Toxins--biosynthesis--BI; Cell Line; Chaperonin 60--biosynthesis--BI; Chaperonin 60--immunology--IM; Cross Reactions; Epithelial Cells; Epithelium--immunology--IM; Epitopes--analysis --AN; Flow Cytometry; Gastric Mucosa--cytology--CY; Gastric Mucosa --immunology--IM; Helicobacter pylori--enzymology--EN; Helicobacter pylori --immunology--IM; Humans; Immunoblotting; Immunohistochemistry; Rabbits; Stomach Neoplasms--immunology--IM; Stomach Neoplasms--pathology--PA; Tumor Cells, Cultured; Urease--analysis--AN
CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antibodies, Monoclonal); 0 (Bacterial Proteins); 0 (Bacterial Toxins); 0 (Chaperonin 60); 0 (Epitopes); 0 (VacA protein, Helicobacter pylori)
Enzyme No.: EC 3.5.1.5 (Urease)
Record Date Created: 19961112

Record Date Completed: 19961112

7/9/22
DIALOG(R)File 155:MEDLINE(R)

11433905 PMID: 8707078
Expression of CD44 and its variants on gastric epithelial cells of patients with Helicobacter pylori colonisation.
Fan X; Long A; Goggins M; Fan X; Keeling P W; Kelleher D
Department of Clinical Medicine, St James's Hospital, Trinity College Dublin, Ireland.
Gut (ENGLAND) Apr 1996, 38 (4) p507-12, ISSN 0017-5749
Journal Code: 2985108R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS
BACKGROUND--Studies have suggested that expression of the adhesion molecule CD44 may be of prognostic importance in gastric cancer. In addition, there is strong evidence that Helicobacter pylori has a role in gastric cancer. AIMS--To determine the expression of CD44 and its variants (v6, v9) and HLA class II molecules on human gastric epithelial cell and intraepithelial lymphocytes in patients with and without H pylori infection. PATIENTS--Eighteen patients (seven men and 11 women) attending for endoscopic evaluation because of upper gastrointestinal symptoms were included. An additional 10 patients (five men and five women) were analysed for CD44 variant expression). METHODS--Biopsy specimens were taken from the gastric antrum during endoscopy. Gastric epithelial cells and intraepithelial lymphocytes were examined by two colour flow cytometry and compared in patients with and without H pylori infection. RESULTS--Expression of CD44 and its variants (CD44 v9) was increased in epithelial cells but not in intraepithelial lymphocytes. Both epithelial cells and intraepithelial lymphocytes expressed higher levels of HLA class II molecules (DR and DP), possibly as a result of local cytokine production. Furthermore, results showed upregulation of CD44 on a gastric epithelial cell line (AGS) by cytokines and peripheral blood mononuclear cell supernatant. CONCLUSIONS--These data suggest that H pylori, either directly or through a local inflammatory response, is responsible for increased expression of CD44 and its variant CD44 v9. These data are of potential importance in relation to increased expression of CD44 and CD44 v9 on gastric carcinoma.
Tags: Female; Male; Research Support, Non-U.S. Gov't
Descriptors: *Antigens, CD44--metabolism--ME; *Gastric Mucosa--immunology --IM; *Helicobacter Infections--immunology--IM; *Helicobacter pylori; *Lymphocytes--immunology--IM; Adult; Aged; Case-Control Studies; Flow Cytometry; Gastric Mucosa--metabolism--ME; Helicobacter Infections --metabolism--ME; Humans; Lymphocytes--metabolism--ME; Middle Aged; Up-Regulation
CAS Registry No.: 0 (Antigens, CD44)
Record Date Created: 19960909
Record Date Completed: 19960909

7/9/23
DIALOG(R)File 155:MEDLINE(R)

11355873 PMID: 8849267
Effect of imipenem on monoclonal antibody-mediated protection against Escherichia coli O18K5.
Frasa H; Benaissa-Trouw B; Tavares L; van Kessel K; Hustinx W; Schellekens J; Kraaijeveld K; Verhoef J
Eijkman-Winkler Institute for Medical Microbiology, University Hospital, Utrecht, The Netherlands.
Antimicrobial agents and chemotherapy (UNITED STATES) Apr 1996, 40 (4) p999-1004, ISSN 0066-4804 Journal Code: 0315061
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Flow cytometry revealed that the binding of immunoglobulin M monoclonal antibodies (MAbs) to Escherichia coli O18K5 was modulated by exposure of the bacteria to subinhibitory concentrations of imipenem. The binding of anti-K5 MAb was decreased, while the binding of anti-O18 MAb was increased. In addition, anti-lipid A MAbs bound only to imipenem-treated bacteria. The biological effect of MAb binding was investigated in BALB/c mice by determination of the levels of bacteremia, tumor necrosis factor (TNF) in serum and survival after intraperitoneal challenge with bacteria preincubated with MAb. Neither MAb alone (150 micrograms per animal) proved to be protective against untreated bacteria. Anti-lipid A MAb on its own, in contrast to anti-K5 and anti-O18 MAbs, was not protective against imipenem-treated bacteria. Only combinations which included anti-O18 MAb and anti-K5 MAb exerted in mice enhanced protection against smooth E. coli O18K5 as well as imipenem-treated E. coli O18K5. This was reflected by reduced TNF levels in serum and increased survival. The addition of anti-lipid A MAb to the combination of anti-K5 MAb and anti-O18 MAb reduced serum TNF levels in mice, but not significantly.
Tags: Comparative Study; Female
Descriptors: *Antibodies, Monoclonal--therapeutic use--TU; *Bacteremia --prevention and control--PC; *Escherichia coli--drug effects--DE; *Imipenem--pharmacology--PD; *Thienamycins--pharmacology--PD; Animals; Antibodies, Monoclonal--immunology--IM; Escherichia coli--immunology--IM; Flow Cytometry; Helicobacter; Mice; Mice, Inbred BALB C
CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Thienamycins); 74431-23-5 (Imipenem)
Record Date Created: 19961024
Record Date Completed: 19961024

7/9/24
DIALOG(R)File 155:MEDLINE(R)

11270295 PMID: 8566853
Helicobacter pylori increases proliferation of gastric epithelial cells.
Fan X G; Kelleher D; Fan X J; Xia H X; Keeling P W
Department of Infectious Diseases, Hsiangya Hospital, Hunan Medical University, China.
Gut (ENGLAND) Jan 1996, 38 (1) p19-22, ISSN 0017-5749 Journal Code: 2985108R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS

The direct and indirect effects of helicobacter pylori on cell kinetics of gastric epithelial cell line AGS were investigated by flow cytometric analysis of Ki-67 positive cells and by MTT assay. Flow cytometric analysis of Ki-67 positivity permits detection of cells that are in S-phase, whereas the MTT assay is a colometric measure of the number of viable cells. In the absence of added stimulants, 23.06 (4.88)% mean (SD) of AGS cells were Ki-67 positive. When cells were preincubated in the presence of H pylori, there was a significant increase in Ki-67 positivity (66.20 (7.89)%, p < 0.001). This increase was not seen in cells cultured in the presence of Campylobacter jejuni (24.63 (8.11)% or Escherichia coli (21.66 (9.78)%). Pre-incubation of AGS cells with supernatants from both H pylori and mitogen activated peripheral blood lymphocytes also increased the per cent of cells that were Ki-67 positive (72.93 (8.68) and 69.96 (12.35)%; p, 0.001) respectively. Similar results were also found in MTT assay. These data show that both H pylori directly and the immune/inflammatory response to H pylori indirectly can influence the rate of epithelial cell proliferation, suggesting this bacterium may be an initiating step in gastric carcinogenesis and an important co-carcinogenic factor in H pylori positive subjects.

Descriptors: *Gastric Mucosa--pathology--PA; *Helicobacter Infections --pathology--PA; *Helicobacter pylori--physiology--PH; *Neoplasm Proteins --metabolism--ME; *Nuclear Proteins--metabolism--ME; Antibodies, Monoclonal ; Cell Division--immunology--IM; Cell Movement; Cells, Cultured; Flow Cytometry; Gastric Mucosa--immunology--IM; Helicobacter Infections --immunology--IM; Humans; Ki-67 Antigen

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Ki-67 Antigen); 0 (Neoplasm Proteins); 0 (Nuclear Proteins)

Record Date Created: 19960304
Record Date Completed: 19960304

7/9/25

DIALOG(R)File 155:MEDLINE(R)

11206791 PMID: 8574746

Adherence of Helicobacter pylori to cultured human gastric carcinoma cells.

Yamamoto-Osaki T; Yamaguchi H; Taguchi H; Ogata S; Kamiya S

Department of Microbiology, Kyorin University, School of Medicine, Tokyo, Japan.

European journal of gastroenterology & hepatology (ENGLAND) Aug 1995, 7 Suppl 1 pS89-92, ISSN 0954-691X Journal Code: 9000874

Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

AIM: To examine the binding activity of Helicobacter pylori to cultured gastric epithelial cells using flow cytometry. MATERIALS AND METHODS: We evaluated the adherence of 15 H. pylori strains to cultured gastric cancer (MKN45) cells by flow cytometric analysis. Other bacterial strains were also analysed for their adherence to MKN45 cells. In addition, we examined the effect of fetuin on the adherence of H. pylori to MKN45 cells. RESULTS: H. pylori strains adhered to MKN45 cells at rates of between 49 and 93.7%, with a mean of 75.3%. In contrast, the rates of Escherichia coli, Shigella flexneri, Vibrio cholerae and Yersinia enterocolitica adherence to MKN45

cells were 69.1, 5.9, 11.7 and 33.1%, respectively. Fetuin had no inhibitory effect on the adherence of H. pylori to MKN45 cells in the flow cytometric analysis. CONCLUSIONS: A flow cytometric analysis using MKN45 cells proved to be an objective and sensitive method for evaluating the adherence of H. pylori, showing close adherence between this organism and gastric epithelial cells.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Helicobacter pylori--isolation and purification--IP; *Stomach Neoplasms--microbiology--MI; *Stomach Neoplasms--pathology--PA; Bacterial Adhesion; Flow Cytometry; Helicobacter pylori--drug effects--DE; Helicobacter pylori--metabolism--ME; Humans; Stomach Neoplasms--metabolism --ME; Tumor Cells, Cultured; alpha-Fetoproteins--pharmacology--PD

CAS Registry No.: 0 (alpha-Fetoproteins)

Record Date Created: 19960313

Record Date Completed: 19960313

7/9/26

DIALOG(R)File 155:MEDLINE(R)

10992397 PMID: 7773247

HLA-DR expression on CD8 lymphocytes from gastric mucosa in urease-positive and urease-negative gastritis.

Ihan A; Krizman I; Ferlan-Marolt V; Tepez B; Gubina M

Institute of Microbiology, Medical Faculty, Ljubljana, Slovenia.

FEMS immunology and medical microbiology (NETHERLANDS) Feb 1995, 10 (3-4) p295-9, ISSN 0928-8244 Journal Code: 9315554

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; AIDS/HIV

We isolated lymphocytes from chronically inflamed gastric mucosa. We analysed the expression of IL-2 receptors (CD25), transferin receptors (CD71) and HLA-DR molecules on T lymphocytes by flow cytometric analysis in 16 patients with urease-positive and in 7 patients with urease-negative chronic gastritis. In G0, G1 and G2 histological type (Sydney classification) of gastritis the number of lymphocytes obtained from the gastric mucosa biopsies was too low for the flow cytometric analysis. However, in G3 histological type of chronic gastritis we obtained enough cells for the flow cytometric analysis in 75%. We demonstrated a significant increase in HLA-DR expression on CD8 cells from patients with urease-positive gastritis compared to urease-negative gastritis. We also observed a statistically non-significant increase in HLA-DR expression on CD3 cells, and in CD71 expression on both CD3 and CD8 cells in urease-positive gastritis. However, no difference in CD25 expression was found between the two types of gastritis.

Tags: Comparative Study; Female; Male

Descriptors: *CD8-Positive T-Lymphocytes--immunology--IM; *Gastritis --immunology--IM; *HLA-DR Antigens--biosynthesis--BI; *Urease--metabolism --ME; Antigens, CD3--biosynthesis--BI; Chronic Disease; Flow Cytometry; Gastric Mucosa--cytology--CY; Gastric Mucosa--immunology--IM; Gastric Mucosa--pathology--PA; Helicobacter Infections--immunology--IM; Helicobacte r pylori--immunology--IM; Helicobacter pylori--pathogenicity--PY; Humans; Lymphocyte Count; Receptors, Interleukin-2--biosynthesis--BI; Receptors, Transferrin--biosynthesis--BI

CAS Registry No.: 0 (Antigens, CD3); 0 (HLA-DR Antigens); 0 (Receptors, Interleukin-2); 0 (Receptors, Transferrin)

Enzyme No.: EC 3.5.1.5 (Urease)
Record Date Created: 19950713
Record Date Completed: 19950713

7/9/27
DIALOG(R)File 155:MEDLINE(R)

10631777 PMID: 8020691
Helicobacter pylori-induced microvascular protein leakage in rats: role of neutrophils, mast cells, and platelets.
Kurose I; Granger D N; Evans D J; Evans D G; Graham D Y; Miyasaka M; Anderson D C; Wolf R E; Cepinskas G; Kvietys P R
Department of Physiology, Louisiana State University Medical Center, Shreveport.
Gastroenterology (UNITED STATES) Jul 1994, 107 (1) p70-9, ISSN 0016-5085 Journal Code: 0374630
Contract/Grant No.: DK 41399; DK; NIDDK; DK 43785; DK; NIDDK; HL26441; HL ; NHLBI
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS
BACKGROUND/AIMS: Previous studies indicate that a water extract of Helicobacter pylori (HPE) can promote neutrophil-endothelial cell interactions in vivo and in vitro. The objectives of this study were to assess whether HPE alters the rate of albumin leakage in rat mesenteric venules and identify the factors that mediate the HPE-induced microvascular dysfunction. METHODS: Intravital microscopy was used to continuously monitor leukocyte adherence and emigration and albumin leakage in rat mesenteric venules during superfusion with HPE. RESULTS: HPE increased leukocyte adherence and emigration and microvascular albumin leakage. The enhanced albumin leak could be subdivided into two components: an early (within 10 minutes) and a later (within 30 minutes) phase. HPE also elicited perivenular mast cell degranulation and the formation of platelet-leukocyte aggregates within post-capillary venules. The HPE-induced early phase of albumin leakage was attenuated by pretreatment with a mast cell stabilizer. The HPE-induced late phase of albumin leakage was reduced by monoclonal antibodies directed against either CD11b/CD18 or intercellular adhesion molecule 1. A monoclonal antibody against P-selectin also inhibited the HPE-induced platelet-leukocyte aggregation and reduced the later phase of albumin leak. CONCLUSIONS: HPE-induced microvascular dysfunction appears to be a consequence of interstitial and intravascular cell-cell interactions.
Tags: Comparative Study; In Vitro; Male; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.
Descriptors: *Albumins--pharmacokinetics--PK; *Blood Platelets --physiology--PH; *Capillaries--cytology--CY; *Capillary Permeability --physiology--PH; *Endothelium, Vascular--cytology--CY; *Helicobacter pylori--physiology--PH; *Mast Cells--physiology--PH; *Neutrophils --physiology--PH; Animals; Blood Platelets--cytology--CY; Capillaries --physiology--PH; Cell Adhesion--physiology--PH; Cell Movement--physiology --PH; Endothelium, Vascular--physiology--PH; Flow Cytometry; Fluorescent Antibody Technique; Helicobacter Infections--blood--BL; Helicobacter Infections--metabolism--ME; Helicobacter Infections--physiopathology--PP; Mast Cells--cytology--CY; Neutrophils--cytology--CY; Rats; Rats, Sprague-Dawley; Venules--cytology--CY; Venules--physiology--PH

CAS Registry No.: 0 (Albumins)
Record Date Created: 19940804
Record Date Completed: 19940804

7/9/28
DIALOG(R)File 155:MEDLINE(R)

09932120 PMID: 1400995
Serological detection of Helicobacter pylori by a flow microsphere immunofluorescence assay.
Best L M; Veldhuyzen van Zanten S J; Bezanson G S; Haldane D J; Malatjalian D A
Department of Microbiology, Victoria General Hospital, Halifax, Nova Scotia, Canada.
Journal of clinical microbiology (UNITED STATES) Sep 1992, 30 (9) p2311-7, ISSN 0095-1137 Journal Code: 7505564
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
A flow cytometric immunofluorescence assay (FMIA) for the detection of immunoglobulin G antibodies to Helicobacter pylori was developed. A multicomponent antigen was prepared and used to coat carboxylated polystyrene microspheres for reaction with patient sera followed by fluorescein isothiocyanate-labelled goat anti-human immunoglobulin G. The reacted microspheres were collected with a flow cytometer, and fluorescence was quantitated relative to the cutoff value provided by pooled sera from patients in whom H. pylori could not be demonstrated by culture or histology. Serum samples from 28 H. pylori-positive patients and 27 H. pylori-negative patients were tested by FMIA. Additionally, an in-house enzyme-linked immunosorbent assay (ELISA) employing the same antigen preparation and a commercially available ELISA were used to assay the patient population. Both the FMIA and in-house ELISA were 100% sensitive and 89% specific with positive and negative predictive values of 90 and 100% and no equivocal results. The commercial ELISA was 96% sensitive and 89% specific with positive and negative predictive values of 90 and 96% and five equivocal results. FMIA provides a rapid, inexpensive, and easily performed means for serodiagnosis of H. pylori.
Tags: Comparative Study; Female; Male
Descriptors: *Antibodies, Bacterial--analysis--AN; *Helicobacter Infections--diagnosis--DI; *Helicobacter pylori--isolation and purification --IP; *Serologic Tests--methods--MT; Adult; Aged; Aged, 80 and over; Antigens, Bacterial--chemistry--CH; Antigens, Bacterial --isolation and purification--IP; Biopsy; Enzyme-Linked Immunosorbent Assay; Evaluation Studies; Flow Cytometry; Fluorescent Antibody Technique; Helicobacter pylori--immunology--IM; Humans; Immunoglobulin G--analysis--AN; Intestinal Mucosa--microbiology--MI; Microspheres; Middle Aged; Predictive Value of Tests; Pyloric Antrum--microbiology--MI; Reagent Kits, Diagnostic
CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Antigens, Bacterial); 0 (Immunoglobulin G); 0 (Reagent Kits, Diagnostic)
Record Date Created: 19921030
Record Date Completed: 19921030

7/9/29
DIALOG(R)File 155:MEDLINE(R)

09871101 PMID: 1499928

Proliferation assay of human gastric remnant by bromodeoxyuridine and flow cytometry.

Ohyama S; Yonemura Y; Miwa K; Miyazaki I; Sasaki T

Second Department of Surgery, School of Medicine, Kanazawa University, Japan.

Gastroenterology (UNITED STATES) Sep 1992, 103 (3) p789-93, ISSN 0016-5085 Journal Code: 0374630

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

The cell kinetics of gastric epithelium were studied by bromodeoxyuridine (BrdU) and flow cytometry in seven patients with remnant stomachs reconstructed with a Billroth type II procedure and in 25 patients with whole stomachs. Each patient received an intravenous injection of BrdU (200 mg/m2) 6 hours before surgery. Fresh specimens obtained from the lesser curvature, greater curvature, and stomal areas in the cases of remnant stomachs and from the antrum and fundus in the case of whole stomachs were studied. The BrdU labeling index was higher in the stomal area of the gastric remnant than in other areas, and DNA synthesis time was shortened in the stomal area of the gastric remnant (P less than 0.01) but not in other areas. The turnover time of the mucosa in the stomal area was 4.1 +/- 1.2 days, significantly shorter (P less than 0.01) than in other areas (7.6 +/- 2.3 to 8.2 +/- 1.2 days). The present study showed that the cell proliferation was extremely rapid in the stomal area of the gastric remnant, suggesting that this enhanced turnover of epithelial cells may assist in promoting carcinogenesis in the stomal area of the gastric remnant.

Descriptors: *DNA--biosynthesis--BI; *Gastrectomy--adverse effects--AE; *Gastric Mucosa--cytology--CY; *S Phase; Aged; Anastomosis, Surgical --adverse effects--AE; Anastomosis, Surgical--methods--MT; Bromodeoxyuridine; Cell Division; Flow Cytometry; Gastric Fundus--cytology--CY; Gastric Fundus--metabolism--ME; Gastric Mucosa--metabolism--ME; Humans; Middle Aged ; Pyloric Antrum--cytology--CY; Pyloric Antrum--metabolism--ME; Time Factors

CAS Registry No.: 59-14-3 (Bromodeoxyuridine); 9007-49-2 (DNA)

Record Date Created: 19920915

Record Date Completed: 19920915

7/9/30

09543534 PMID: 1925306

Adherence of Helicobacter pylori to gastric carcinoma cells: analysis by flow cytometry.

Dunn B E; Altmann M; Campbell G P

Laboratory Service, Denver Veterans Administration Medical Center, Colorado.

Reviews of infectious diseases (UNITED STATES) Jul-Aug 1991, 13 Suppl 8 pS657-64, ISSN 0162-0886 Journal Code: 7905878

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

An in vitro assay using immunofluorescence and flow cytometry for quantitative assessment of the adherence of Helicobacter pylori to cultured human gastric carcinoma (KATO III) cells was developed. Adherence was rapid, saturable, energy dependent, mannose resistant, and significantly inhibited by fetuin, a glycoprotein containing N-acetylneuraminyllactose. Pretreatment of KATO cells with neuraminidase from Clostridium perfringens, however, did not reduce adherence of H. pylori. Ultrastructurally, adherent cells of H. pylori were associated with indentations of KATO cell membranes. KATO cells should prove useful in the investigation of mechanisms of adherence of H. pylori to mammalian cells. Ultimately, this flow cytometric assay may be helpful in assessment of the adherence of laboratory strains of H. pylori directly to surface mucous cells dissociated from biopsied human gastric tissue.

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.

Descriptors: *Bacterial Adhesion; *Helicobacter pylori--metabolism--ME; Bacterial Adhesion--drug effects--DE; Cell Line; Flow Cytometry; Fluorescent Antibody Technique; Helicobacter pylori--ultrastructure--UL; Humans; Microscopy, Electron; Microscopy, Fluorescence; Stomach Neoplasms; Tumor Cells, Cultured

Record Date Created: 19911120
Record Date Completed: 19911120
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$6.30 30 Type(s) in Format 9
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$11.67 Estimated cost File155
$0.80 INTERNET
$12.47 Estimated cost this search
$12.49 Estimated total session cost 1.866 DialUnits
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              785  S4
      S5      880  (S1 OR S2) AND (S3 OR S4)
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              880  S5
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            20714  PYLORI
              178  PYLORIDIS
               10  PYLORIS
      S6        2  S5 AND (HELICOBACT? OR PYLORI OR PYLORIDIS OR PYLORIS)
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T S6/9/ALL

  6/9/1
DIALOG(R)File 155:MEDLINE(R)
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13462442   PMID: 10430335
 Diminished interferon-gamma production in gastric mucosa T lymphocytes
 after H. pylori eradication in duodenal ulcer patients.
  Ihan A; Tepez B; Gubina M; Malovrh T; Kopitar A
  Institute of Microbiology, Medical Faculty, Ljubljana, Slovenia.
IHAN@.MF.UNI-LJ.SI
  Hepato-gastroenterology (GREECE) May-Jun 1999, 46 (27) p1740-5,
ISSN 0172-6390 Journal Code: 8007849
  Publishing Model Print
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: MEDLINE; Completed
  Subfile: INDEX MEDICUS
  BACKGROUND/AIMS: Helicobacter pylori (H. pylori) infects an estimated 50%
of the world population; however, only a small proportion of individuals
develop clinical symptoms of gastritis, peptic ulceration or gastric
cancer. The variations in disease presentation may be due to differences in
bacterial virulence and/or immune response to the pathogen. In a previous
study we reported an increased expression of the IL-2 receptor in duodenal
ulcer (DU) patients. The present study examines the expression of IL-2
receptor and intracellular lymphokine production in gastric mucosa
infiltrating T lymphocytes in DU patients before and after H. pylori
eradication. METHODOLOGY: T lymphocytes were isolated from gastric mucosa
biopsies by using mechanical and enzymatic tissue desegregation.
Ficoll-purified lymphocytes were incubated with monoclonal antibodies and
analyzed by using 4-color flow cytometry analysis for the IL-2 receptor
(CD25) and intracellular interferon-gamma (IFN-gamma) and IL-4 expression.
Lymphocytes from 24 H. pylori-infected patients with severe gastric mucosa
infiltration (G2 and G3 histological type in Sydney classification) were
analyzed. RESULTS: We demonstrated a significant decrease in IL-2 receptor
expression on gastric mucosa T cells 3 and 12 months after eradication of
H. pylori. We also demonstrated a diminished IFN-gamma production 3 and 12
months after H. pylori eradication. CONCLUSIONS: Our results suggest that
cellular immune activation in gastric mucosa is reversibly dependent on the
presence of H. pylori.
  Descriptors: *Anti-Bacterial Agents--therapeutic use--TU; *Duodenal Ulcer
--drug therapy--DT; *Gastric Mucosa--drug effects--DE; *Helicobacter
Infections--drug therapy--DT; *Helicobacter pylori--drug effects--DE;
*Interferon Type II--metabolism--ME; Amoxicillin--adverse effects--AE;
```

7/9/6
DIALOG(R)File 155:MEDLINE(R)

12619650 PMID: 10535482

The vast majority of gastric T cells are polarized to produce T helper 1 type cytokines upon antigenic stimulation despite the absence of Helicobacter pylori infection.

Itoh T; Wakatsuki Y; Yoshida M; Usui T; Matsunaga Y; Kaneko S; Chiba T; Kita T

Department of Clinical Bio-regulatory Science, Kyoto University Graduate School of Medicine, Japan.

Journal of gastroenterology (JAPAN) Oct 1999, 34 (5) p560-70, ISSN 0944-1174 Journal Code: 9430794

Publishing Model Print; Comment in J Gastroenterol 1999 Oct;34(5) 651-2; Comment in PMID 10535499

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; AIDS/HIV

Helicobacter pylori infection is associated with chronic infiltration by various cell types, including T cells, whose cytokine production may regulate the inflammatory reaction as well as local immune response to the bacterium. We prospectively analyzed the constituents of the cellular infiltrates and the cytokines produced by T cells in antral biopsies obtained from 73 subjects with and without H. pylori infection, before and after eradication therapy, and compared them with a histological grade of gastritis. We found that T cells predominated in cell number, followed by granulocytes/monocytes and plasma cells in both H. pylori-infected and H. pylori-uninfected subjects. Despite the absence of H. pylori infection, more than 70% of gastric CD4-positive T cells obtained from uninfected tissue produced interferon-gamma (IFN-gamma) in the cytosol. Upon receptor cross-linking of a CD3 and a CD28 molecule, T cells in both infected and uninfected tissue continuously secreted a far greater amount of IFN-gamma than those in peripheral blood mononuclear cell controls for a period of cell culture, whereas the increase in interleukin-4 (IL-4) was very small, and no increase in IL-2 secretion was seen. In H. pylori-infected patients, IFN-gamma secretion was correlated with the grade of mononuclear cell infiltration and decreased to an uninfected control level after eradication therapy. We did not see the effect of eradication on IL-4 secretion. Anti-H. pylori antibody of the IgG2 subclass was remarkably increased in H. pylori-infected subjects. These results together suggest that gastric T cells are already differentiated to produce a large amount of IFN-gamma by a mechanism unrelated to H. pylori infection. H. pylori infection appeared to activate T cells to secrete even more IFN-gamma, which may contribute to maintaining a perpetual inflammation in H. pylori-infected stomach.

Tags: Female; Male; Research Support, Non-U.S. Gov't

Descriptors: *Gastritis--immunology--IM; *Helicobacter Infections --immunology--IM; *Interferon - Type II--biosynthesis--BI; *Stomach --immunology--IM; *Th1 Cells--immunology--IM; Adult; Aged; Antibodies, Bacterial--biosynthesis--BI; Biopsy; Coculture Techniques; Flow Cytometry; Gastritis--microbiology--MI; Gastritis--pathology--PA; Helicobacter Infections--pathology--PA; Helicobacter pylori--immunology--IM; Humans; Immunoglobulin G--biosynthesis--BI; Interleukin-2--biosynthesis--BI; Interleukin-4--biosynthesis--BI; Lymphocyte Activation; Middle Aged; Prospective Studies; Statistics, Nonparametric; Stomach--pathology--PA

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Immunoglobulin G); 0 (Interleukin-2); 207137-56-2 (Interleukin-4); 82115-62-6 (Interferon

--UL; Humans; Immunoblotting; Interleukin-8--secretion--SE; Microscopy, Electron, Scanning
CAS Registry No.: 0 (Antacids); 0 (Chaperonin 60); 0 (Drug Combinations); 0 (Interleukin-8); 1309-42-8 (Magnesium Hydroxide); 21645-51-2 (Aluminum Hydroxide); 37317-08-1 (aluminum magnesium hydroxide)
Record Date Created: 19991021
Record Date Completed: 19991021

7/9/4
DIALOG(R)File 155:MEDLINE(R)

13462442 PMID: 10430335
Diminished interferon-gamma production in gastric mucosa T lymphocytes after H. pylori eradication in duodenal ulcer patients.
Ihan A; Tepez B; Gubina M; Malovrh T; Kopitar A
Institute of Microbiology, Medical Faculty, Ljubljana, Slovenia. IHAN@.MF.UNI-LJ.SI
Hepato-gastroenterology (GREECE) May-Jun 1999, 46 (27) p1740-5, ISSN 0172-6390 Journal Code: 8007849
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
BACKGROUND/AIMS: Helicobacter pylori (H. pylori) infects an estimated 50% of the world population; however, only a small proportion of individuals develop clinical symptoms of gastritis, peptic ulceration or gastric cancer. The variations in disease presentation may be due to differences in bacterial virulence and/or immune response to the pathogen. In a previous study we reported an increased expression of the IL-2 receptor in duodenal ulcer (DU) patients. The present study examines the expression of IL-2 receptor and intracellular lymphokine production in gastric mucosa infiltrating T lymphocytes in DU patients before and after H. pylori eradication. METHODOLOGY: T lymphocytes were isolated from gastric mucosa biopsies by using mechanical and enzymatic tissue desegregation. Ficoll-purified lymphocytes were incubated with monoclonal antibodies and analyzed by using 4-color flow cytometry analysis for the IL-2 receptor (CD25) and intracellular interferon-gamma (IFN-gamma) and IL-4 expression. Lymphocytes from 24 H. pylori-infected patients with severe gastric mucosa infiltration (G2 and G3 histological type in Sydney classification) were analyzed. RESULTS: We demonstrated a significant decrease in IL-2 receptor expression on gastric mucosa T cells 3 and 12 months after eradication of H. pylori. We also demonstrated a diminished IFN-gamma production 3 and 12 months after H. pylori eradication. CONCLUSIONS: Our results suggest that cellular immune activation in gastric mucosa is reversibly dependent on the presence of H. pylori.
Descriptors: *Anti-Bacterial Agents--therapeutic use--TU; *Duodenal Ulcer --drug therapy--DT; *Gastric Mucosa--drug effects--DE; *Helicobacter Infections--drug therapy--DT; *Helicobacter pylori--drug effects--DE; *Interferon Type II--metabolism--ME; Amoxicillin--adverse effects--AE; Amoxicillin--therapeutic use--TU; Anti-Bacterial Agents--adverse effects --AE; Drug Therapy, Combination; Duodenal Ulcer--immunology--IM; Duodenal Ulcer--pathology--PA; Flow Cytometry; Follow-Up Studies; Gastric Mucosa --immunology--IM; Gastric Mucosa--pathology--PA; Helicobacter Infections --immunology--IM; Helicobacter Infections--pathology--PA; Helicobacter pylori--immunology--IM; Humans; Interleukin-4--analysis--AN; Metronidazole

Amoxicillin--therapeutic use--TU; Anti-Bacterial Agents--adverse effects --AE; Drug Therapy, Combination; Duodenal Ulcer--immunology--IM; Duodenal Ulcer--pathology--PA; Flow Cytometry; Follow-Up Studies; Gastric Mucosa --immunology--IM; Gastric Mucosa--pathology--PA; Helicobacter Infections --immunology--IM; Helicobacter Infections--pathology--PA; Helicobacter pylori--immunology--IM; Humans; Interleukin-4--analysis--AN; Metronidazole --adverse effects--AE; Metronidazole--therapeutic use--TU; Organometallic Compounds--adverse effects--AE; Organometallic Compounds--therapeutic use --TU; Receptors, Interleukin-2--analysis--AN; Receptors, Interleukin-2 --drug effects--DE; T-Lymphocytes--drug effects--DE; T-Lymphocytes --immunology--IM; T-Lymphocytes--pathology--PA; Treatment Outcome

CAS Registry No.: 0 (Anti-Bacterial Agents); 0 (Organometallic Compounds); 0 (Receptors, Interleukin-2); 207137-56-2 (Interleukin-4); 26787-78-0 (Amoxicillin); 443-48-1 (Metronidazole); 57644-54-9 (bismuth tripotassium dicitrate); 82115-62-6 (Interferon Type II)

Record Date Created: 19990922

Record Date Completed: 19990922

6/9/2

DIALOG(R)File 155:MEDLINE(R)

12619650 PMID: 10535482

The vast majority of gastric T cells are polarized to produce T helper 1 type cytokines upon antigenic stimulation despite the absence of Helicobacter pylori infection.

Itoh T; Wakatsuki Y; Yoshida M; Usui T; Matsunaga Y; Kaneko S; Chiba T; Kita T

Department of Clinical Bio-regulatory Science, Kyoto University Graduate School of Medicine, Japan.

Journal of gastroenterology (JAPAN) Oct 1999, 34 (5) p560-70, ISSN 0944-1174 Journal Code: 9430794

Publishing Model Print; Comment in J Gastroenterol. 1999 Oct;34(5) 651-2; Comment in PMID 10535499

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; AIDS/HIV

Helicobacter pylori infection is associated with chronic infiltration by various cell types, including T cells, whose cytokine production may regulate the inflammatory reaction as well as local immune response to the bacterium. We prospectively analyzed the constituents of the cellular infiltrates and the cytokines produced by T cells in antral biopsies obtained from 73 subjects with and without H. pylori infection, before and after eradication therapy, and compared them with a histological grade of gastritis. We found that T cells predominated in cell number, followed by granulocytes/monocytes and plasma cells in both H. pylori-infected and H. pylori-uninfected subjects. Despite the absence of H. pylori infection, more than 70% of gastric CD4-positive T cells obtained from uninfected tissue produced interferon-gamma (IFN-gamma) in the cytosol. Upon receptor cross-linking of a CD3 and a CD28 molecule, T cells in both infected and uninfected tissue continuously secreted a far greater amount of IFN-gamma than those in peripheral blood mononuclear cell controls for a period of cell culture, whereas the increase in interleukin-4 (IL-4) was very small, and no increase in IL-2 secretion was seen. In H. pylori-infected patients, IFN-gamma secretion was correlated with the grade of mononuclear cell infiltration and decreased to an uninfected control level after eradication therapy. We did not see the effect of eradication on IL-4 secretion.

Anti-H. pylori antibody of the IgG2 subclass was remarkably increased in H. pylori-infected subjects. These results together suggest that gastric T cells are already differentiated to produce a large amount of IFN-gamma by a mechanism unrelated to H. pylori infection. H. pylori infection appeared to activate T cells to secrete even more IFN-gamma, which may contribute to maintaining a perpetual inflammation in H. pylori-infected stomach.

Tags: Female; Male; Research Support, Non-U.S. Gov't

Descriptors: *Gastritis--immunology--IM; *Helicobacter Infections --immunology--IM; *Interferon Type II--biosynthesis--BI; *Stomach --immunology--IM; *Th1 Cells--immunology--IM; Adult; Aged; Antibodies, Bacterial--biosynthesis--BI; Biopsy; Coculture Techniques; Flow Cytometry; Gastritis--microbiology--MI; Gastritis--pathology--PA; Helicobacter Infections--pathology--PA; Helicobacter pylori--immunology--IM; Humans; Immunoglobulin G--biosynthesis--BI; Interleukin-2--biosynthesis--BI; Interleukin-4--biosynthesis--BI; Lymphocyte Activation; Middle Aged; Prospective Studies; Statistics, Nonparametric; Stomach--pathology--PA

CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Immunoglobulin G); 0 (Interleukin-2); 207137-56-2 (Interleukin-4); 82115-62-6 (Interferon Type II)

Record Date Created: 19991102

Record Date Completed: 19991102

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E2        1       INTERLEUKIN 31 RECEPTOR, MOUSE
E3        0      *INTERLEUKIN 4
E4        1       INTERLEUKIN 4 (1-129)-PE38KDEL
E5       11       INTERLEUKIN 4 (38-37)-PE38KDEL
E6      327       INTERLEUKIN 5 RECEPTOR
E7       19       INTERLEUKIN 6-INTERLEUKIN 6 RECEPTOR FUSION PR
E8        2       INTERLEUKIN 8 (77)
E9        2       INTERLEUKIN 8 (77), ALA-
E10      53       INTERLEUKIN 9 RECEPTOR
E11   28708   12  INTERLEUKIN-1
E12     731       INTERLEUKIN-1 --ADMINISTRATION AND DOSAGE --AD

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E2        0    1  INTERLEUKIN-3 RECEPTORS
E3    12690   13 *INTERLEUKIN-4
E4      175       INTERLEUKIN-4 --ADMINISTRATION AND DOSAGE --AD
E5       30       INTERLEUKIN-4 --ADVERSE EFFECTS --AE
E6        5       INTERLEUKIN-4 --AGONISTS --AG
E7        5       INTERLEUKIN-4 --ANALOGS AND DERIVATIVES --AA
E8      572       INTERLEUKIN-4 --ANALYSIS --AN
E9      308       INTERLEUKIN-4 --ANTAGONISTS AND INHIBITORS --A
E10    2997       INTERLEUKIN-4 --BIOSYNTHESIS --BI
E11     680       INTERLEUKIN-4 --BLOOD --BL
E12       8       INTERLEUKIN-4 --CEREBROSPINAL FLUID --CF

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S E3-E12
           12690  INTERLEUKIN-4
             175  INTERLEUKIN-4 --ADMINISTRATION AND DOSAGE --AD
              30  INTERLEUKIN-4 --ADVERSE EFFECTS --AE
               5  INTERLEUKIN-4 --AGONISTS --AG
               5  INTERLEUKIN-4 --ANALOGS AND DERIVATIVES --AA
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             308  INTERLEUKIN-4 --ANTAGONISTS AND INHIBITORS --A
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               8  INTERLEUKIN-4 --CEREBROSPINAL FLUID --CF
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E14     113  INTERLEUKIN-4 --CHEMISTRY --CH
E15     196  INTERLEUKIN-4 --DEFICIENCY --DF
E16       2  INTERLEUKIN-4 --DIAGNOSTIC USE --DU
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E18    1335  INTERLEUKIN-4 --IMMUNOLOGY --IM
E19      22  INTERLEUKIN-4 --ISOLATION AND PURIFICATION --I
E20    1339  INTERLEUKIN-4 --METABOLISM --ME
E21       9  INTERLEUKIN-4 --PHARMACOKINETICS --PK
E22    3347  INTERLEUKIN-4 --PHARMACOLOGY --PD
E23    1158  INTERLEUKIN-4 --PHYSIOLOGY --PH
E24       3  INTERLEUKIN-4 --RADIATION EFFECTS --RE

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E25     544       INTERLEUKIN-4 --SECRETION --SE
E26       3       INTERLEUKIN-4 --STANDARDS --ST
E27     179       INTERLEUKIN-4 --THERAPEUTIC USE --TU
E28      18       INTERLEUKIN-4 --TOXICITY --TO
E29       1       INTERLEUKIN-4 --URINE --UR
E30     916       INTERLEUKIN-4 //RECEPTORS, (RECEPTORS, INTERLEUKIN-4)
E31       0    1  INTERLEUKIN-4 RECEPTORS
E32       5       INTERLEUKIN-4 STAT
E33       1       INTERLEUKIN-4 VARIANT
E34       2       INTERLEUKIN-4-INDUCED PROTEIN, FIND
E35       5       INTERLEUKIN-4-PSEUDOMONAS EXOTOXIN
E36    3491    7  INTERLEUKIN-5

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            196  INTERLEUKIN-4 --DEFICIENCY --DF
              2  INTERLEUKIN-4 --DIAGNOSTIC USE --DU
           1920  INTERLEUKIN-4 --GENETICS --GE
           1335  INTERLEUKIN-4 --IMMUNOLOGY --IM
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           1339  INTERLEUKIN-4 --METABOLISM --ME
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              3  INTERLEUKIN-4 --RADIATION EFFECTS --RE
            544  INTERLEUKIN-4 --SECRETION --SE
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             18  INTERLEUKIN-4 --TOXICITY --TO
              1  INTERLEUKIN-4 --URINE --UR
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Ref   Items   RT  Index-term
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E3    52727    8 *FLOW CYTOMETRY
E4        4       FLOW CYTOMETRY --ADVERSE EFFECTS --AE
E5        2       FLOW CYTOMETRY --CLASSIFICATION --CL
E6       36       FLOW CYTOMETRY --ECONOMICS --EC
E7        1       FLOW CYTOMETRY --ETHICS --ES
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E8       20        FLOW CYTOMETRY --HISTORY --HI
E9      908        FLOW CYTOMETRY --INSTRUMENTATION --IS
E10    6524        FLOW CYTOMETRY --METHODS --MT
E11       1        FLOW CYTOMETRY --NURSING --NU
E12     381        FLOW CYTOMETRY --STANDARDS --ST

          Enter P or PAGE for more
?

S E3-E12
           52727  FLOW CYTOMETRY
               4  FLOW CYTOMETRY --ADVERSE EFFECTS --AE
               2  FLOW CYTOMETRY --CLASSIFICATION --CL
              36  FLOW CYTOMETRY --ECONOMICS --EC
               1  FLOW CYTOMETRY --ETHICS --ES
              20  FLOW CYTOMETRY --HISTORY --HI
             908  FLOW CYTOMETRY --INSTRUMENTATION --IS
            6524  FLOW CYTOMETRY --METHODS --MT
               1  FLOW CYTOMETRY --NURSING --NU
             381  FLOW CYTOMETRY --STANDARDS --ST
      S3   52727  E3-E12
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Ref    Items    RT  Index-term
E13      125       FLOW CYTOMETRY --STATISTICS AND NUMERICAL DATA
E14       50       FLOW CYTOMETRY --TRENDS --TD
E15        4       FLOW CYTOMETRY --UTILIZATION --UT
E16      606       FLOW CYTOMETRY --VETERINARY --VE
E17      981     1 FLOW INJECTION ANALYSIS
E18        2       FLOW INJECTION ANALYSIS --ECONOMICS --EC
E19      194       FLOW INJECTION ANALYSIS --INSTRUMENTATION --IS
E20      402       FLOW INJECTION ANALYSIS --METHODS --MT
E21       13       FLOW INJECTION ANALYSIS --STANDARDS --ST
E22       12       FLOW INJECTION ANALYSIS --STATISTICS AND NUMER
E23        4       FLOW INJECTION ANALYSIS --TRENDS --TD
E24        2       FLOW INJECTION ANALYSIS --VETERINARY --VE

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?

S E13-E16
            125  FLOW CYTOMETRY --STATISTICS AND NUMERICAL DATA
             50  FLOW CYTOMETRY --TRENDS --TD
              4  FLOW CYTOMETRY --UTILIZATION --UT
            606  FLOW CYTOMETRY --VETERINARY --VE
      S4    785  E13-E16
?


Set      Items   Description
S1       12690   E3-E12
S2        8505   E13-E29
S3       52727   E3-E12
S4         785   E13-E16
?

S (S1 OR S2) AND (S3 OR S4)
           12690  S1
            8505  S2
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1: Rinsho Byori. 1998 Dec;46(12):1247-51. Related Articles, Books, LinkOut

[Flow cytometric analysis of helper T cell subsets (Th1 and Th2) in healthy adults]

[Article in Japanese]

Tanaka K, Kemmotsu K, Ogawa K, Ishii N, Minami M, Nagata K, Takano S.

R & D Center, BML Inc., Kawagoe.

To estimate frequency of T helper (Th) 1, Th2, and other related subsets in T cells among healthy Japanese, we determined the frequencies of interferon (IFN)-gamma- and/or interleukin (IL)-4-producing cells in stimulated peripheral blood lymphocytes (PBL) from 51 healthy adults of various ages by three-color flow cytometry using FITC-labeled anti-IFN-gamma, PE-conjugated anti-IL-4, and peridinin chlorophyll protein (PerCP)-labeled anti-CD4 or anti-CD3 antibody. As a result, proportions (mean +/- SD) of IFN-gamma-single positive (conveniently called Th1), IL-4-single positive (Th2), and double-positive cells (Th0) in CD4+ PBL, and homologous cell populations in CD3+ PBL (Th1 type, Th2 type, and Th0 type cells) were 21.3 +/- 8.2%, 2.7 +/- 1.1%, 1.9 +/- 1.0%, 39.5 +/- 13.5%, 1.7 +/- 0.8%, and 1.8 +/- 1.0%, respectively. Percentages of Th1 and Th2 were roughly consistent with those reported previously. Proportions of Th1, Th2, Th1 type, and Th2 type cells were found to increase with the age, whereas there were no significant difference in sex at least in 20s. Furthermore, positive correlation was seen between Th1 and Th1 type ($r = 0.79$), Th2 and Th2 type ($r = 0.87$), and Th0 and Th0 type ($r = 0.86$). Frequencies of these subsets in each donor were maintained at substantially the same levels for at least 3 months, suggesting existence of particular and stable Th1/Th2 balance among healthy individuals. As reported, 6 patients with atopic dermatitis examined in this study showed significantly lower frequencies of Th1 (11.0 +/- 2.5%) as compared with healthy donors ($p < 0.01$), implying usefulness of the immune test.

PMID: 9916512 [PubMed - indexed for MEDLINE]

Help

Exp. Anim. 49(2), 97–110, 2000

Upgrading of Flow Cytometric Analysis for Absolute Counts, Cytokines and Other Antigenic Molecules of Cynomolgus Monkeys (Macaca fascicularis) by Using Anti-Human Cross-Reactive Antibodies

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Abstract: *In order to effectively use cynomolgus monkeys as animal models for human diseases, more than 300 anti-human monoclonal antibodies (mAbs) were studied as to their cross-reaction with various antigens from cynomolgus monkeys (Macaca fascicularis). Two hundred twenty-nine of 339 (67.55%) anti-human mAbs that react with human antigens of CD-defined molecules, chemokine receptors, and T cell receptors were cross-reactive with the monkey antigens. Using the cross-reactive antibodies and the fluorescenced beads for calibration, the procedure for the absolute count of monkey lymphocyte subsets was developed and the mean values for $CD4^+$ and $CD8^+$ lymphocyte subsets in peripheral blood were 718 and 573/mm^3, respectively. Moreover, intracellular cytokines, IL-2, IL-4 and IFNγ, and intracellular apoptosis-related proteins, Bcl-2, FADD and active form of caspase-3 could be detected in peripheral blood mononuclear cells as well as various tissue cells. It is therefore practicable to detail the phenotype of leukocytes, assess the production of intracellular cytokines and enumerate T-lymphocyte subsets by using the cross-reactive human antibodies with respective antigens of cynomolgus monkeys.*

Key words: *cross-reactive anti-human mAb, cynomolgus monkey, flow cytometry*

Introduction

Monkeys of the macaque species, which include cynomolgus (*Macaca fascicularis*), rhesus (*Macaca mulatta*) and pig-tailed monkey (*Macaca nemestrina*) are invaluable experimental animals in biomedical research, because the macaque species are phylogenetically proximate to human. Actually, the structure of biologically relevant molecules in monkeys and the organization of macaque's hematopoietic and immune systems are nearly identical to those of man. Furthermore, it is possible to study the acute, subacute and

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chronic toxicity of an antiretrovirus [3, 11, 44], the pathogenesis of infectious diseases [27], the hematological changes caused by long term exposure to various reagents [45], the immune dysfunction by viral infections [52], allogenic transplantation [30, 35], cancer gene therapy [40], aging [62] and stress [47] by using the monkey models. These features make macaques unique as models for various human diseases and it becomes possible to understand the mechanisms of pathogenesis and efficacy of a variety of prophylactic and therapeutic agents including vaccines and antiretrovirals by preclinical trials with the monkey models.

For analysis of the animals used in the models, efforts have been made to phenotype the leukocyte and hematopoietic progenitor cells, detection of both red blood cell (RBC) and platelet markers, and detection of intracellular proteins including various cytokines and regulatory molecules. Although some monoclonal antibodies (mAb), e.g. that are specific for the CD3 antigen of cynomolgus monkeys (anti-cynomolgus monkey CD3 mAb, 6G12) [25] or rhesus monkeys (anti-rhesus monkey CD3 mAb, FN18) [36], were developed, the numbers of available antibodies specific for macaque antigens are too few to study the animals. Recently, anti-human mAbs which were used to define the lineage and function of leukocyte subsets and cytokines in man, have been shown to cross-react with the homologous determinants on macaque antigens by using flow cytometry [2, 19, 32, 39, 43, 50, 53, 58], immunoprecipitation [2, 7], bioassay [29, 46], enzyme-linked immunosorbent assay [23] and immunohistochemistry [31, 54, 63], but they are still not enough even to determine the distribution of functional details of the subsets of monkey leukocytes. In this study, we evaluated 339 anti-human mAbs for the cross-reactivity with cynomolgus monkey antigens by using three color flow cytometry.

Materials and Methods

Animals: Cynomolgus monkeys kept in the breeding colony of Tsukuba Primate Center, National Institute of Infectious Diseases (NIID), Japan, were used according to the guidelines of the NIID and were free of known simian retroviruses, herpesviruses, bacteria and parasites. The study was conducted with the approval of an institutional committee for P3 level animal experiments and in accordance with the requirements specifically stated in the laboratory biosafety manual of the World Health Organization in the P3 facility for monkeys in Murayama Branch, NIID, Musashimurayama, Tokyo, Japan. Each animal was anesthetized with ketamine (Sankyo Co., Ltd., Tokyo, Japan). Blood samples obtained from healthy cynomolgus monkeys or normal human volunteers were collected by venipuncture with anticoagulant EDTA-2Na or heparin.

Antibodies: The anti-human mAbs tested in this study are listed in Table 1. These mAbs were obtained from Beckman Coulter (Miami, FL), Becton Dickinson (San Jose, CA), BioSource (Camarill, CA), Caltag (Burlingame, CA), DAKO (Copenhagen, Denmark), Endogen (Woburn, MA), Nichirei (Tokyo, Japan), Ortho (Raritan, NJ), Pharmingen (San Diego, CA), R&D Systems (Minneapolis, MN), Serotec (Kidlington, Oxford, UK) and Transduction Laboratories (Lexington, KY). For indirect cell staining, unlabeled anti-human mAbs were detected with phycoerythrin (PE)-conjugated goat anti-mouse IgG mAb $(Fab')^2$ (Beckman Coulter), PE-conjugated goat anti-mouse IgM mAb $(Fab')^2$ (Beckman Coulter) or PE-conjugated goat anti-rat IgG mAb $(Fab')^2$ (Beckman Coulter). All anti-human mAbs were tested at pretitrated concentrations.

Cell preparation and staining: Tissues and peripheral blood were harvested by using a sterile technique and observing appropriate biohazard precautions. Portions of tissues were dissociated to liberate single cells. For cell surface leukocyte antigen analysis, RBC in peripheral blood were lysed by ACK lysis buffer (distilled water with 0.826% of NH_4Cl, 0.1% of $KHCO_3$ and 0.0037% of EDTA-2Na, pH 7.3). Peripheral blood was mixed with lysis buffer (peripheral blood: lysis buffer = 1:14) and left for 5 min at room temperature. After centrifugation at 300 × g for 5 min, the cells were washed with PBS and the living cells were counted by a trypan blue dye exclusion method. For platelet preparation, platelet-rich plasma was prepared by centrifugation at 200 × g for 15 min. Bone marrow cells in femur were prepared by lysed RBC. The cells were incubated with each anti-human mAb for 30 min and then washed with staining buffer three times [64]. For activation of PBMC, each 500 μl of hepalinized peripheral blood was placed in a 12 × 75-mm polystyrene tube (Becton Dickinson) and mixed with an

Table 1. Crossreaction of anti-human mAb with cynomolgus monkey antigen

Antigen	Source[a]	Clone	Crossreactivity[b]
CD1a	BD	SK9	+
	D	NA1/34	+
	BC	SFCI19Thy1A8(T6)	–
CD1b	BC	4A7.6	+
CD2	BC	SFCI3Pt2H9	+
	BC	39C1.5	+
	BD	S5.2	+
	D	MT910	+
	N	Nu-Ter	+
	P	RPA-2.10	+
CD3	D	T3-4B5	+
	P	SP34	+
	BS	CRIS-7	(+)
	BC/D/P	UCTH1	–
	BD	SK7	–
	N	NU-T3	–
	P	HIT3a	–
CD4	BD	SK3	+
	D	MT310	+
	N	NU-TH/I	+
	BC	SFCI12T4D11(T4)	–
	BC	13B8.2	–
	P	RPA-T4	–
CD4 v4	BD	L120	–
CD5	C	CD5-5D7	+
	BC	BL1a	–
	BC	SFCI24T6G12(T1)	–
	D	DK23	–
CD6	BC	SPLV14	+
	P	M-T605	+
	S	F10-205-11	+
CD7	P	M-T701	+
	BC	3A1E-12H7	–
	BD	4H9	–
	D	DK24	–
CD8	BD	SK1	+
	D	DK25	+
	N	NU-TS/C	+
	P	RPA-T8	+
	S	YTC182.20	+
	S	YTC141.1	+
	BC	SFCI21Thy2D3(T8)	–
	BC	B9.11	–
CD8β	BC	5F2	+
	BC	2ST8.5H7	+
CD9	D	P1/33/2	+
	P	M-L13	+
	BC	ALB6	–
CD10	BC	ALB1	+
	BC	ALB2	+
	BC	J5	+
	D	SS2/36	+
	P	HI10a	+
CD11a	BC	25.3	+
	BD	G-25.2	+
	P	HI111	+
	D	MHM24	–
CD11b	BC	94(Mo1)	+
	BC	Bear1	+
	BD	D12	+
	P	ICRF(44)	+
	S	ICRF44	+
	D	2LPM19c	–
CD11c	BC	BU15	+
	BD	S-HCL-3	+
	S	3.9	+
	D	KB90	–
CD13	N	MCS-2	+
	BC	366(MY7)	–
	D	WM-47	–
CD14	BC	116(Mo2)	+
	BC	322A-1(MY4)	+
	BC	RMO52	+
	BD	MfP9	+
	D	TUK4	+
	N	CLB-Mon/1	+
	P	M5E2	+
	S	UCHM-1	+
CD15	BC	80H5	–
	P	HI98	–
CD16	BC/P	3G8	+
	BD	NKP15	+
CD16b	BC	1D3	–
CD18	BC	7E4	+
	BD	L130	+
	D	MHM23	+
CD19	BC	J4.119	(+)
	BC	89B(B4)	–
	BD	4G7	–
	D	HD37	–
	P	B43	–
	P	HIB19	–
CD20	BC	B9E9(HRC20)	+
	BD	L27	+
	D	B-Ly1	+
	N	NU-B2	+
CD21	BC	BL13	+
	P	B-ly4	+
	BC	B120	–

(continued)

Antigen	Source[a]	Clone	Crossreactivity[b]
CD23	BC	9P25	+
	D	MHM6	+
	P	M-L233	+
CD25	BC	IHT44H3(IL-2R1)	+
	BC	33B3.1	+
	BD	2A3	+
	BD	M-A251	+
	BS	B-B10	+
	D	ACT-1	+
	BC	B1.49.9	–
CD26	BC	4EL-1C7(Ta1)	–
	BC	BA5	–
	P	M-A261	–
CD27	BC	1A4CD27	+
	P	M-T271	+
	S	LT27	+
CD28	BC/P	CD28.2	+
	BD	L293	+
	D	CD28.1	+
	N	KOLT-2	+
CD29	BC	4B4LD9LDH8	+
	BC/D	K20	+
	P	MAR4	+
	S	Jb1B	+
CD30	BC	HRS4	+
	D	Ber-H2	+
CD31	BC	5.6E	+
	BD	L133.1	+
	P	WM-59	+
CD32	BC	2E1	+
CD33	BC	906(MY9)	–
	D	WM-54	–
CD34	BC/D	QBEnd10	+
	BC	581	+
	N	NU-4A1	+
CD35	BC	J3D3	+
	P/S	E11	+
CD36	BC	FA6.152	+
CD37	BC	BL14	–
	D	HH1	–
CD38	O	OKT10	+
	BC	T16	–
	D	AT13/5	–
	N/P	HIT2	–
	BC	AC2	+
CD39	P	Tu66	+
CD40	BC	MAB89	+
	P	5C3	+
	S	B-B20	+
CD41	D	5B12	+
CD43	D	DF-T1	–
	P	IG10	–

(continued)

Antigen	Source[a]	Clone	Crossreactivity[b]
CD44	BC	J.173	+
	D	DF1485	+
	P	G44-26	+
	S	MCA89F	+
CD45	D	2B11+PD7/26	+
	BC	ALB12	–
	BC	IMMU19.2	–
	BC	J.33	–
	BC	B3821F4A	–
	BD	2D1	–
	D	T29/33	–
	P	HI30	–
CD45RA	BC	2H4LDH11LDB9	+
	BC	ALB11	+
	BD	L48	+
	D	4KB5	+
	P	HI100	–
CD45RB	D	PD7/26	+
CD45RO	BC/BD/D/N/P	UCHL-1	(+)
CD46	BC	J4.48	+
	P	E4.3	–
CD47	P	CIKM1	+
CD48	BC	J4.57	–
CD49b	BC	Gi9	+
	P	12F1-H6	+
	S	AK7	+
CD49d	BC	HP2/1	+
	BD	L25	+
	P	9F10	+
	S	HP2/1	+
CD49e	BC	SAM1	+
	P	VC5	+
CD49f	BC/P	GoH3	+
CD50	BC	HP2/19	–
	P	TU41	–
CD52	S	YTH66.9	+
CD53	P	HI29	–
CD54	C	MEM111	+
	BD	LB-2	–
	BC	84H10	–
	D	6.5B5	–
	P	HA58	–
CD56	BC	N901	+
	BC	T199	+
	BD	MY31	+
	N	NKI-nbl-1	+
	P	B159	+
CD57	BD	HNK-1	–
	BC/N	NC1	–
CD58	BD	L306.4	+

(continued)

Antigen	Source[a]	Clone	Crossreactivity[b]
CD62L	BD	SK11	+
	D	FMC46	+
	BC	SFCI128T17G6(TQ1)	–
	BC	DREG56	–
CD63	BC	CLBGran/12	+
CD64	BC	22	+
	P	10.1	+
	S	10.1	+
CD65	BC	88H7	–
CD66b	BC	80H3	–
CD68	D	KP1	+
CD69	BC	TP1.55.3	+
	BD	L78	+
	D/P	FN50	+
CD70	BC	HNE51	+
CD71	D	Ber-T9	+
	BC	YDJ1.2.2	–
CD78	P	FN1	+
CD79b	BC/P	CB3-1	–
	D	SN8	–
CD80	BC	MAB104	+
	BD	L307.4	+
	P	BB1	–
CD81	BC	JS64	+
	P	JS-81	+
CD83	BC	HB15a	+
CD86	P	2331(FUN-1)	+
CD87	P	VIM5	–
CD89	BC	A3	+
	P	A59	+
CD90	BC	F15-42-1-5	+
	P	5E10	+
CD94	BC	HP-3B1	–
CD95	BD/D/P	DX2	+
	BC	CH11	–
	BC	7C11	–
	BC	UB2	–
CD95L	C	alf-2.1	+
	M	4A5	+
	M	4H9	+
CD97	P	VIM3b	–
CD100	P/S	A8	+
CD101	P	V7.1	+
CD102	BC	BT-1	–
CD103	BC	2G5	+
	D	Ber-ACT8	+
CD106	BC	1G11	–
CD116	BC	SCO6	+
CD117	N	NU-c-kit	+
	BC	95C3	+
	D	104D2	+
CD119	C	MMHGR-1	+

(continued)

Antigen	Source[a]	Clone	Crossreactivity[b]
CD120a	R	16803.1	+
	C	2H10	–
CD120b	D	22235.3	+
	C	4D1B10	–
CD122	N	Mik-β1	+
	P	Mik-β2	+
	BC	2RB(IL-2R(p75))	–
CD123	P	7G3	+
CD124	BC	S456C9	–
CD125	P	A14	+
CD126	BC	M91	–
CD127	BC	R34.34	+
CD135	BC	SF1.340	+
CD138	BC	BB4	–
CD152	P	BNI3	–
CD154	BC/D/P	TRAP1	–
CD158a	BC	EB6	–
CD158b	BC	GL183	–
CD162	P	KPL-1	+
TCR$\alpha\beta$	S	R73	+
	S	8A3	+
	BC	BMA031	–
	BD	WT31	–
	P	T10B9.1A-31	–
TCR$\gamma\delta$	E	5A6.E9	+
	BC	IMMU510	–
	BD	11F2	–
	P	B1.1	–
	BC	IMMU515	–
	S	F1	+
	BC	C15	+
TCRVβ1	BC	BL37.2	+
TCRVβ2	BC	MPB2D5	–
TCRVβ3	BC	CH92	–
TCRVβ5.1	BC	IMMU157	+
TCRVβ5.2	BC	36213	+
TCRVβ5.3	BC	3D11	–
TCRVβ7	BC	ZOE	–
TCRVβ8	BC	56C5.2	–
TCRVβ9	BC	FIN9	+
TCRVβ11	BC	C21	–
TCRVβ12	BC	VER2.32.1	+
TCRVβ13.1	BC	IMMU222	–
TCRVβ13.6	BC	JU74.3	+
TCRVβ14	BC	CAS1.1.3	–
TCRVβ16	BC	TAMAYA1.2	–
TCRVβ17	BC	E17.5F3.15.13	+
TCRVβ18	BC	BA62.6	–
TCRVβ20	BC	ELL1.4	+
TCRVβ21.3	BC	IG125	–
TCRVβ22	BC	IMMU546	–
TCRVβ23	BC	AF23	+

(continued)

Antigen	Source[a]	Clone	Crossreactivity[b]
TCRVγ9	BC	IMMU360	+
	P	B3.1	+
TCRVδ1	S	TS8.2	+
TCRVδ2	BC	IMMU389	+
	P	B6	+
TCRVδ3	BC	P11.5B	+
HLA-ABC	BC	B9.12.1	+
	S	W6/32	+
	S	BB7.1	+
HLA-DR	BC	B8.12.2	+
	BD	L243	+
	P	Tu36	+
HLA-DP,DQ,DR	BC	9-49(I3)	+
	D	CR3/43	+
CCR1	D	53504.111	+
CCR5	P	2D7/CCR5	+
CCR5	R	45531.111	+
	R	45549.111	+
CCR6	R	53103.111	+
CXCR2	D	48311.211	+
CXCR3	D	49801.111	+
CXCR4	P/R	12G5	+
CXCR5	D	51505.111	+
Integrin β7	P	FIB504	+
p38	BC	C1.7	–
IL-10R	P	3F9	+
IL-12Rβ1	P	TOS	+
IL-2	BC	N7.48A	+
	P	MQ1-17H12	+
IL-4	P	8D4-8	+
IFN-γ	BC	45.15	+
	P	4S.B3	+
Bcl-2	D	124	+
FADD	T	1	+
active form of caspase-3[c]	P		+

[a] BC, Beckman Coulter; BD, Becon Dickinson; BS, BioSource; C, Caltag; D, DAKO; E, Endogen; N, Nichirei; O, Ortho; P, Pharmingen; R, R&D Systems; S, Serotec; T, Transduction Laboratories. [b] +, positive reaction; –, negative reaction; (+), weak staining. [c] rabbit polyclonal Ab.

appropriate volume of RPMI 1640 medium. Then the diluted samples were incubated with 0.25 μg/m*l* of phorbol 12-myristate 13-acetate (PMA; Sigma, St. Louis, MO) and 1 μg/m*l* of ionomycin (Sigma) for 4 hr at 37°C. Each 100 μ*l* of activated blood sample was incubated with anti-human mAbs for 30 min at room temperature. RBC was lysed with FACS lysing solution (Becton Dickinson) for 10 min at room temperature. All samples were analyzed with a FACSCalibur (Becton Dickinson) equipped with an argon laser tuned at 488 nm and with Cell Quest software (Becton Dickinson).

Absolute counts of peripheral T-lymphocyte by flow cytometry: Monkey peripheral blood was collected with EDTA-2Na. Fifty microliters of blood was placed in a 12 × 75-mm polystyrene tube and stained with FITC-conjugated anti-monkey CD3 mAb (FN18; BioSource), PE-conjugated anti-human CD4 mAb (SK3; Becton Dickinson) and peridinin chlorophyll protein (PerCP)-conjugated anti-human CD8 mAb (SK1; Becton Dickinson) for 15 min at 4°C. The blood was lysed with FACS lysing solution for 15 min at 4°C. After adding 50 μ*l* of Flow Count (Beckman Coulter), 1 × 10^4 FN18 positive cells were analyzed with a FACSCalibur.

Intracellular cytokine staining: For intracellular cytokine staining, each 500 μ*l* of hepalinized peripheral blood was placed in a 12 × 75-mm polystyrene tube and mixed with an appropriate volume of RPMI 1640 medium. The diluted samples were then incubated with 0.25 μg/m*l* of PMA, 1 μg/m*l* of ionomycin and 10 μg/m*l* of brefeldin A (Sigma) for 4 hr at 37°C. Each 100 μ*l* of activated blood sample was incubated with mAbs against cell surface antigens for 30 min at room temperature. RBC was lysed with FACS lysing solution for 10 min at room temperature. The cells were permeabilized with FACS permeabilizing solution (Becton Dickinson) for 10 min at room temperature. After washing three times, the permeabilized cells were incubated with mAbs for cytokine or CD95L (Fas ligand; clone alf-2.1, PE-conjugated) for 30 min at room temperature. All samples were analyzed with a FACSCalibur and Cell Quest software.

Active form of caspase-3 staining: For the active form of caspase-3 staining, T-2 toxin (a trichothecene mycotoxin [59]), which was kindly donated by Dr. Y. Ueno (Institute of Tochigi Clinical Pathology), was used for the induction of apoptosis [37, 60, 65]. T-2 toxin treated cells were fixed with FACS lysing solution for 10 min at room temperature. The cells were permeabilized by FACS permeabilizing solution for 10 min at room temperature. After washing three times, the permeabilized cells were incubated with PE-conjugated anti-active form of caspase-3 polyclonal Ab for 30 min at room temperature. All samples were analyzed with a FACSCalibur and Cell Quest software.

Apoptosis induction by anti-human CD95 mAbs:

Human and monkey PBMC were isolated by density-gradient centrifugation from heparinaized peripheral blood. PBMC were cultured in RPMI 1640 supplemented with 10% FCS, 5 μg/ml of phytohemagglutinin P (PHA-P; DIFCO Lab., Detroit, MI) and antibiotics for 48 hr. The PHA-stimulated PBMC were cultured with anti-human CD95 mAb (CH11, 7C11 or DX2) 48 hr. By using DX2, the cells were cultured with or without 2 μg/ml of Protein G (Sigma). Apoptotic nuclei were measured by flow cytometry with propidium iodide (PI) staining. The method for quantitative analysis of apoptosis was described previously [64].

Results

Cross-reactivity of anti-human antibodies with cynomolgus antigens

Three hundred thirty-nine anti-human mAbs from 11 companies were first screened against cynomolgus monkey antigens of CD-defined molecules, chemokine receptors, T cell receptors, intracellular cytokines and intracellular apoptosis-related proteins. In summary, 229 anti-human mAbs (67.55%) were crossreacted with cynomolgus monkey antigens (Table 1).

Almost all commercially available mAbs recognizing CD-defined antigens were tested on peripheral blood cells freshly obtained from normal healthy animals. Anti-CD1a and CD41 mAbs reacted with thymocytes and platelets, respectively. Moreover, we found clones of anti-human CD13, CD33, CD34, CD117 (also called c-kit) and CD135 (flt3/flk2) mAbs that reacted with monkey bone marrow cells but did not find cross-reactive anti-human CD33 mAb. Although the clones of anti-human CD102 (ICAM-2) and CD106 (VCAM-1) used in this study were not cross-reactive with monkey endothelial cells from abdominal aorta, those anti-human adhesion molecule mAbs that were noncommercially available were reported to be cross-reactive [51, 55]. Furthermore, anti-human CD103 mAbs recognizing human integrin αIEL chain was found to cross-react with mucosal tissue lymphocytes from lamina propria (LPL) and intraepithelium (IEL) in monkey intestines (Fig. 1). Altogether, although we newly discovered many cross-reactive anti-human CD antibodies by using 339 antibodies in the study, the noncross-reactive CD-defined antigens on the surface of cynomolgus monkey cells still remained to continued to be pursued as a matter of course (Table 1).

Monoclonal antibodies against human CD19 (J4.119) and CD45RO (UCHL-1) stained weakly with cynomolgus monkey lymphocytes. And there were non-crossreactive antigens of CD15, CD26, CD33, CD43, CD50, CD57, CD79b, CD152 and CD154. It is of interest that a monkey antigen of CD90 (Thy 1) is expressed on leukocytes (Fig. 2), but not on human leukocyte. Nevertheless, CD90 was expressed on the surface of hematopoietic stem cells, nerve cells, and high endothelial venule cells of the lymph node in man [12, 57]. In cynomolgus monkey, CD90 was mainly strongly expressed on T-lymphocytes and natural killer (NK) cells and slightly on B-lymphocytes in monkey PBL. And CD56, which is known as the prototypic marker of human NK cells, was expressed on monocytes but not on NK cells [data not shown].

The human mAbs against monkey T cell receptors were screened with peripheral T-lymphocytes from 3 normal cynomolgus monkeys, 5 *Mycobacterium bovis* bacillus Calmette-Guérin-vaccinated cynomolgus monkeys and 5 hepatitis B virus core antigen-inoculated cynomolgus monkeys. As shown in Table 1, there were only 18 reactive antibodies out of 34, which seems to be not enough to study the T cell receptor repertoire in the cynomolgus monkey models. On the other hand, almost all chemokine receptors which were screened with PBLs of cynomolgus monkeys cross-react with anti-human chemokine receptor mAbs (Table 1).

Absolute count of leukocyte subsets using the cross-reactive anti-human antibodies

The conventional approach used to obtain an absolute T-cell subset count demands data from two different instruments, i.e. a flow cytometer and a hematology analyzer. With the conventional method, there is the possibility of error accumulation from multiple sources from combined procedures for the cell count. On the other hand, a count procedure which uses calibrated beads with fluorescent properties allows volumetric measurement of T-cell subsets in whole blood and can avoid the error accumulation [10]. With the calibrated beads method, the absolute cell count by flow cytometry was developed and this single-platform absolute cell count technology was applied to monkey studies. In 68 normal cynomolgus monkeys tested, the $CD3^+$ PBL were gated and the mean of the $CD4^+$ and $CD8^+$

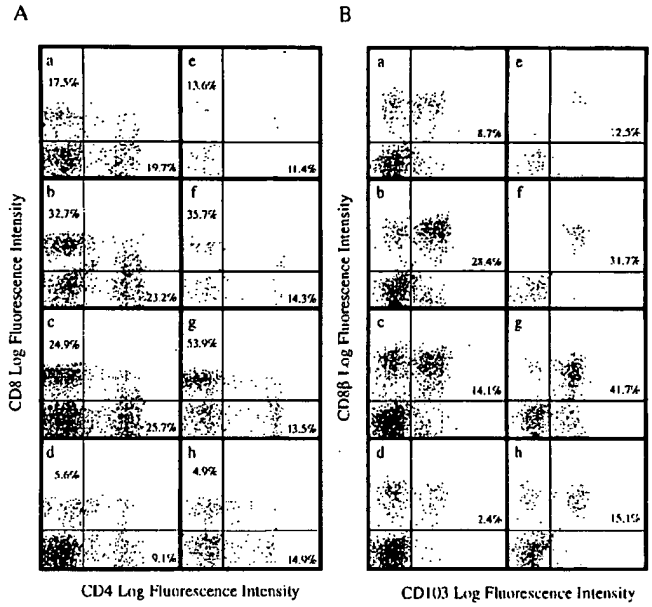

Fig. 1. Expression of adhesion molecule CD103 on mucosal lymphocytes in the cynomolgus monkey detected by cross-reactive human antibodies to CD103. Lymphocytes were stained with CD4/CD8 (A) and CD8β/CD103 (B) following isolation from LP (a–d) and IE (e–h) in the duodenum (a, e), jejunum (b, f), ileum (c, g) and descending colon (d, h). Fluorescence gatings are set to include lymphocyte fraction and relative percentage of each quadrant are shown.

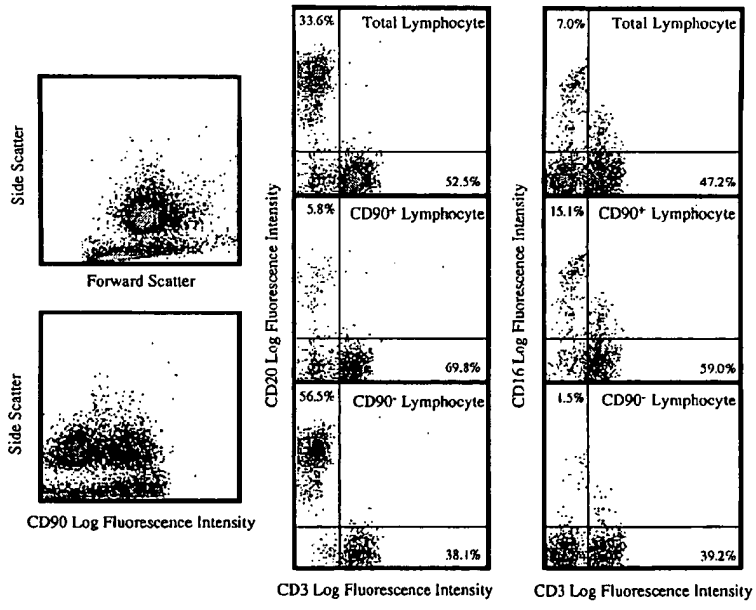

Fig. 2. Representative flow cytometric plots of peripheral blood lymphocytes in the cynomolgus monkey for CD20$^+$ or CD16$^+$ in CD90$^+$ T-lymphocytes. Leukocytes were shown by using linear forward scatter and 90° side scatter (upper left) and 90° side scatter and log CD45 fluorescence (lower left). Leukocytes were stained with CD3/CD20/CD90 (middle panel) and CD3/CD16/CD90 (right panel). Fluorescence gatings are set to include lymphocyte fraction.

T-lymphocyte counts were 718 ± 286 and 673 ± 362 cells/mm^3, respectively.

Detection of intracellular cytokines and apoptosis-related proteins in monkeys cells

The recent identification of anti-cytokine mAbs suitable for staining intracellular cytokines has provided the tools for multiparameter flow cytometric analysis of cytokine-producing cells within unseparated cell populations [16, 24, 42]. Staining for one cell surface antigen and two cytoplasmic cytokines has been used to identify and enumerate cell types which express cytokines in a restricted manner, for example Th1 versus Th2 cells, or an unrestricted manner, for example Th0 cells. As shown in Table 2 and Fig. 3, interleukin-2 (IL-2), IL-4 and interferon-γ(IFNγ) were devised to be detected in PBL of cynomolgus monkey and revealed that IL-2, IFNγ and IL-2/IFNγ were predominant in normal healthy animals, suggesting the Th1 immunological state of the animals.

Moreover, detection of intracellular molecules of apoptosis-related proteins such as CD95L (Fig. 4), Bcl-2 and Fas-associated death domain protein (FADD) was also possible by cross-reacting anti-human mAb with monkey antigens (Table 1). Caspase-3, recently proved as the major protease in apoptotic signal transduction, was detected as an active form in T-2 toxin induced monkey PBMC [data not shown]. We also tested whether anti-human CD95 mAbs bind to monkey CD95 receptor, because CD95 starts to play a role in the programmed sequence of events leading to cell death by cross-linking with its ligand or some of the mAbs [22, 56]. Monkey PBMCs were stimulated with PHA for 48 hr and the blasted cells were targeted for bindability with anti-human CD95 mAbs, CH11, 7C11 and DX2. As shown in Table 1, CH11 and 7C11 antibodies did not cross-reacted with monkey PBMC; only DX2 antibody reacted with the monkey PBMC, but the DX2 antibody was not able to induce apoptosis in PHA-stimulated monkey PBMC in the presence of protein G, although the antibody induced enhanced apoptosis in human PHA-stimulated PBMC by coculturing the human cells with the antibody in the presence of protein G (data not shown).

Table 2. Intracellular cytokine in lymphocyte from cynomolugus monkeys

cytokine	mean ± SD[a)]	
IL-2	12.52 ± 3.76	n = 22
IL-4	1.93 ± 1.28	n = 22
IFNγ	13.09 ± 9.22	n = 22
IL-2$^+$ IFNγ^-	9.56 ± 3.25	n = 22
IL-2$^+$ IFNγ^+	2.96 ± 1.12	n = 22
IL-2$^-$ IFNγ^+	10.31 ± 8.86	n = 22

[a)] percentages of positive cells were analyzed in CD2 positive gated lymphocytes

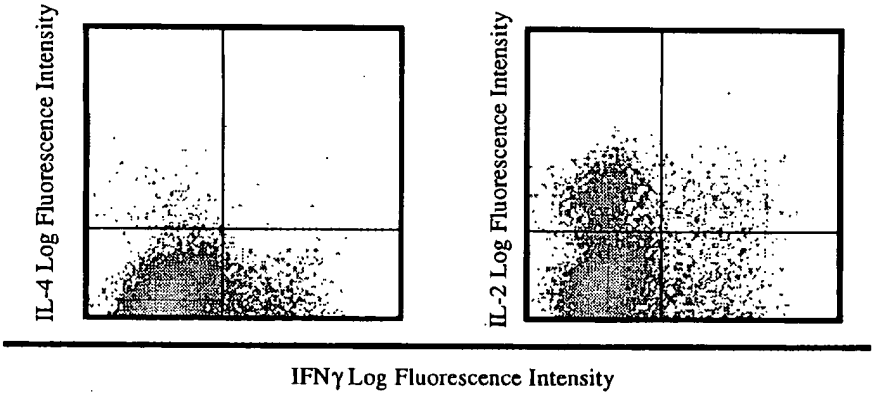

Fig. 3. Flow cytometric plots of peripheral blood lymphocytes in the cynomolgus monkey to detect intracellular cytokine productions. The T cell gate was set by using linear 90° side scatter and log CD2 fluorescence and then T-lymphocytes were defined as CD2^{bright} with low side scatter. Lymphocytes were stained with IFNγ/IL-4/CD2 (left panel) and IFNγ/IL-2/CD2 (right panel) under stimulation by PMA and ionomycin.

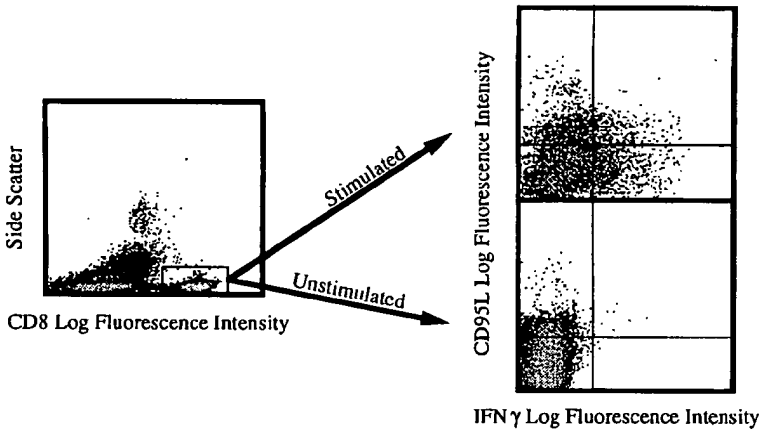

Fig. 4. Enhanced expression of CD95L and IFNγ in peripheral blood lymphocytes of the cynomolgus monkey by stimulation with PMA and ionomycin. The T cell gate was set by using linear 90° side scatter and log CD8 fluorescence and then T-lymphocytes was defined as $CD8^{bright}$ with low side scatter. Lymphocytes were stained with IFNγ/CD95L/CD8 under stimulation by PMA and ionomycin (left panel) as described in *Materials and Methods* and unstimulation (right panel).

Discussion

More than 300 mAbs raised against human leukocyte surface molecules, cytokines or cytoplasmic regulatory molecules have been used to detect antigenically homologous structures of cynomolgus monkey antigens both on the cell surface and in the cytoplasm of PBL and/or other lymphoid tissue cells. We found that approximately 68% of the anti-human mAb tested were positive for crossreactivity with the respective cynomolgus antigens, indicating that leukocyte's subsets can be defined in PBL and various lymphoid tissues of cynomolgus monkeys by selecting the clones of commercially available antibodies. Although the bindability of anti-human antibodies to cynomolgus monkey lymphocytes varies on clones used for the studies, many of the T and B cell determinants on the cell surface were conserved and their immunological phenotypes were able to be defined precisely.

It is of interest that expressions of some antigens on the leukocytes are differently distributed in man and monkeys, e.g. CD90 antigen defined by anti-human CD90 mAb binds to monkey $CD3^+$ lymphocytes, the expression pattern of which is identical with its antigen expression on mouse lymphocytes. In contrast, human CD90 is not expressed on lymphocyte but is on hematopoietic stem cells and nerve cells, and it has been estimated that CD90 may contribute to regulating the proliferation and differentiation of hematopoietic stem cells and memory formation of neurons in the central nervous system. Since CD90 antigen is a signal inducible protein on mouse, the functions of CD90 on monkey leukocytes might be similar to those of mouse CD90, but not to human CD90. CD56 antigen defined by human anti-CD56 mAb was expressed on monocyte in both cynomolgus and rhesus macaques [5], but not on human monocytes. In man it binds with NK cells that express $CD3^-$ TCR^- (α, β, γ, δ) large granular lymphocytes and is co-expressed with $CD16^+$ antigen on the cells [20].

Though CD19 and CD45RO are major antigens for phenotyping the B cells and memory cells in cynomolgus monkeys, respectively, their antigens are usually stained weakly on the cell surface, and it requires amplification to detect the antigens [15]. Alternatively, their phenotypings were usually studied by using different antibodies, e.g. with CD20 antigens instead of CD19 as a B cell marker and combining CD45RA and CD62L, or CD29 as memory cell marker, which methods are common in monkey studies [4, 8, 33, 49].

Among the non-crossreactive cynomolgus antigens, some were considered to be important but still remained

to be clarified. For instance, they are CD26 antigen which is an associated marker of autoimmune diseases, adenosine deaminase-deficiency and HIV pathogenesis in humans [18, 38], CD152 antigen which is proved to be primarily a negative regulator of T cell activation [28], and p38 (C1.7) antigen which is expressed as a novel signal transduction molecule on human cytotoxic T-lymphocytes [41, 61]. Moreover, only half of the anti-human mAbs against the variable region of T cell receptors were cross-reacted with that of the monkey TCR. In monkey TCR analysis, the combination of cytometric and genetic molecular analysis is now needed to study the T cell receptor repertoire.

On the other hand, antibodies to human chemokine receptors were screened by using monkey peripheral blood leukocytes and it was found that all of tested animals clearly cross-reacted with monkey chemokine receptors. These molecules that are essential for inflammatory or host-defense responses to infections appear to readily cross-react between humans and nonhuman primates. Recently chemokine receptors were reported to serve as coreceptors for the entry of several pathogens, e.g. human immunodeficiency virus (HIV) and plasmodium [13, 14, 21]. From this point of view, a cross-reactive study of the antigenicity of the molecules demonstrates that many monkey responses are analogous to human responses, which suggests that the monkey is suitable as a model animal for biological responses or diseases in humans.

With regard to the absolute counts of leukocytes, non-human primate models have been developed to study infectious diseases, and accurate counting of leukocytes subsets is crucial to understanding the infection of pathogens, such as simian/human immunodeficiency virus (SHIV). In HIV-infected individuals, the $CD4^+$ T-lymphocyte count is one of the important markers for determining disease progression [17] and the procedure for enumeration of human CD4 and CD8 T-lymphocyte was recently developed with the recommendation that the panel should be a three color combination requiring two tubes. They were with CD45/CD3/CD4 and CD45/CD3/CD8 for three color protocols [34]. In this protocol, lymphocyte gate was set by using linear 90° side scatter and log CD45 fluorescence and then lymphocytes were defined as $CD45^{bright}$ with low side scatter. In this study, we developed an absolute count procedure for monkeys. Since there were no mAbs against monkey CD45 in this study, T-lymphocyte gate, not lymphocyte gate was set by using linear 90° side scatter and log CD3 fluorescence so that the T-lymphocyte purity of the gate becomes close to 100%. Moreover, cynomolgus monkey has significant levels of CD4 and CD8 double positive T-lymphocytes in PBL [1]. It is therefore necessary to stain CD4 and CD8 antigens at the same time. With this procedure, even low cynomolgus T-lymphocyte counts were accurately determined with the CD3/CD4/CD8 staining combination.

The ability to assess intracellular functional markers by multiparameter flow cytometry offers some unique advantages in analyzing the immune system in monkeys. Cytokines produced by Th1 and Th2 cells play a key role in determining the balance between these two immunologic outcomes and the relationship appears to be important in HIV infection where prior Th1 or Th2 biases influence either initial viral susceptibility or progression to AIDS through immune activation [9, 48]. We made it possible to detect cytokine-producing monkey cells by using the cross-reactive anti-human cytokine Abs. Furthermore, CD95L induces apoptosis via its receptor and is released from the cell surface by matrix metalloproteinases (MMP) like tumor necrosis factor (TNF) [26], and it is difficult to detect cell surface CD95L directly, without using MMP inhibitor in *ex vivo*. This detection of CD95L in $CD8^+$ T lymphocytes may become a new index for apoptosis studies and also for detection of frequency of activated cytotoxic T lymphocytes. Moreover, intracellular apoptosis-related proteins, Bcl-2, FADD and an active form of caspase-3 were able to be detected in monkey cells. It therefore seems to be practicable to measure intracellular molecules *in vivo* in nonhuman primate models by using multicolor flow cytometry.

In the present study anti-human CD95 mAbs were tested for their cross-reactivity. Though DX2 antibody cross-reacted with monkey PBMC and cross-linking with this mAb by protein G delivers an apoptotic signal to human CD95 positive cells [6], this mAb could not induce apoptosis on PHA-activated monkey lymphocytes. Although it is not clear how this difference in bindability and signal transduction between monkey and man affects the induction of apoptosis, the structure of the functional epitope of monkey CD95 recognized by DX2 antibody might be different from the human

epitope.

Altogether although non-crossreactive antigens and some differences in biological actions of mAbs on monkey cells remain, it is practicable to use the cross-reactive human mAbs with cynomolgus antigens by upgrading multicolor flow cytometric analysis in non-human primate models. The methods are effective for detailing the phenotype of leukocytes, detecting the production of intracellular cytokines and enumerating T-lymphocyte subsets.

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Flow Cytometric Measurement of Intracellular Cytokines Detects Immune Responses in MUC1 Immunotherapy[1]

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ABSTRACT

The detection of tumor-specific T cells in immunized cancer patients usually relies on lengthy and difficult CTL assays; we now report on flow cytometry to detect the intracellular cytokines interleukin 2 (IL-2), IL-4, IFN-γ, and tumor necrosis factor α (TNF-α) produced by $CD4^+CD69^+$ and $CD8^+CD69^+$ activated T cells after MUC1 antigen stimulation. Peripheral blood mononuclear cells were obtained from 12 patients with adenocarcinoma injected with mannan-MUC1; cells were exposed *in vitro* for 18 h to MUC1 peptide in the presence of CD28 monoclonal antibody and Brefeldin; permeabilized cells were used for the expression of cytokines. After stimulation *in vitro* with MUC1-variable number of tandem repeats peptides, $CD8^+CD69^+$ T cells from all immunized patients generated 3–9 times higher levels of TNF-α ($P < 0.038$) and IFN-γ ($P < 0.010$) than did cells from 12 normal subjects; minor increases in IL-4 occurred. By contrast, $CD4^+CD69^+$ cells showed no overall alteration in TNF-α and IFN-γ cytokine production, although in some patients, their measurement was informative; the measurement of IL-2 was not useful in either $CD4^+CD69^+$ or $CD8^+CD69^+$ cells. We conclude that in MUC1-immunized patients, the measurement of TNF-α and IFN-γ in activated $CD69^+CD8^+$ T cells may be indicative of their immune status.

INTRODUCTION

Most immunotherapeutic studies for solid tumors are attempting to induce $CD8^+$ CTLs rather than antibodies for antitumor affects, and they include the use of dendritic cells pulsed in culture and reinfused (1–3), targeting the mannose receptor with oxidized mannan-conjugated MUC1 peptides (4), and peptides given with adjuvants or cytokines encapsulated in liposomes (5–9). In these studies, measurement of the immune status of mice is straightforward, but it is difficult to measure CTLs in the peripheral blood of patients compared to using the spleen of mice. Limiting dilution assays to measure the CTLp[4] requires prolonged restimulation *in vitro* with Ag and IL-2. However after up to five rounds of restimulation, the relationship between CTLs originally present *in vivo* and what is subsequently found in culture is obscure (10, 11). In clinical studies, the difficulties are further increased, particularly when dealing with patients with advanced cancer in Phase I studies. For the identification of CTLs in diseases such as breast and colon cancer, in which nonimmunized patients do not usually have preexisting CTLs (12), the aim is to induce CTLs rather than merely increase their frequency, although in melanoma, CTLs can be found in nonimmune individuals (13). Thus, the clinical measurement of CTLs and CTLp is difficult and time consuming and yields results of doubtful significance. It is therefore important in tumor immunotherapy to have methods that are simple and that objectively measure the immunization response.

Recently, in infectious disease and tumor immunotherapy, several new approaches have been introduced to quantitatively measure cellular immune responses, such as the detection of secreted cytokines by Elispot assays (14, 15), HLA tetramer binding studies (16–18), and the measurement of intracellular cytokines by flow cytometry (19–21). Because the quantitative assay of intracytoplasmic cytokine production has been used with success in viral infection, wherein both memory and effector T cell responses were found, we used this assay to assess the immune status of cancer patients. We now report that after immunization with mannan MUC1, patients have activated ($CD69^+$) $CD8^+$ T cells that produce IFN-γ and TNF-α.

MATERIALS AND METHODS

Ags. Human MUC1-GST fusion protein containing five VNTR regions of the sequence PAHGVTSAPDTRPAPGSTAP was expressed in *Escherichia coli*, purified and chemically conjugated to mannan to form M-FP (22). GST was cleaved from the fusion protein using the site-specific protease factor Xa obtained from Roche Molecular Biochemicals (Mannheim, Germany), and the fusion protein containing the five VNTR lacking GST was labeled as VNTR. Tetanus toxoid and influenza vaccine (A/Johannesburg/82/96, A/Sydney/5/97, and B/Harbin/7/

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[4] The abbreviations used are: CTLp, CTL precursor frequency; Ag, antigen; APC, allophycocyanin; BDIS, Becton Dickinson Immunocytometry Systems; BFA, Brefeldin A; IL, interleukin; GST, glutathione *S*-transferase; mAb, monoclonal antibody; M-FP, mannan-MUC1 fusion protein; PBMC, peripheral blood mononuclear cell; PE, phycoerythrin; PerCP, peridinin chlorophyl protein; PHA, phytohemagglutinin; TNF, tumor necrosis factor; VNTR, variable number of tandem repeats.

94), obtained from CSL Parkville, Australia, were dialyzed overnight in PBS to remove the preservatives and kept at −20°C in PBS.

Antibodies. The following mAbs were obtained from BDIS (San Jose, CA) conjugated to either FITC, PE, PerCP, or APC: Leu4 (CD3; PerCP and APC), Leu3a (CD4; PerCP and APC), Leu2a (CD8; PerCP and APC), Leu23 (CD69; PE and PerCP), Leu28 (CD28; nonconjugated), IFN-γ (clone 25723.11; FITC and PE), TNF-α (clone 6401.1111; FITC and PE), IL-2 (clone 5344.111; FITC and PE), IL-4 (clone 3010.211; PE), γ1 (mouse IgG1 control; FITC and PE), and γ2α (mouse IgG2α control; FITC and PE).

Cell Preparation and Antigenic Stimulation. PBMCs were obtained from whole blood that was collected from the following: (*a*) normal subjects; (*b*) subjects boosted with tetanus toxoid or influenza vaccine; or (*c*) patients with adenocarcinoma injected i.m. with M-FP, 1 week after their last M-FP immunization. Patients received a total of seven immunizations. Blood was collected in CPT blood collection tubes obtained from Becton Dickinson Vacutainer Systems (Franklin Lakes, NJ), and PBMCs were separated by centrifugation. PBMCs (3×10^7) were placed in 16 × 125-mm polystyrene tissue culture tubes, 3 μg of CD28 mAb were added, and the cultures were left for 10 min at room temperature. Ags and PHA were added at previously determined optimal concentrations (50 μg/ml M-FP, 20 μg/ml VNTR, 10 μg/ml influenza vaccine, 10 μg/ml tetanus toxoid, 2 μg/ml PHA), and the tubes were placed at a 5° horizontal slant at 37°C in a humidified 10% CO_2 incubator for 18 h, with BFA added at a final concentration of 5 μg/ml after 2 h. BFA is a potent inhibitor of intracellular transport that results in intracellular accumulation of cytokines. Because of the toxicity associated with prolonged exposure to BFA, we observed no decrease in the viability of cells at the concentration of BFA used, which was lower than that described in other methods employing a 10-h incubation with BFA. PHA rather than PMA/ionomycin (23) was used as an indicator of positive stimulation because cells could survive with no apparent loss in viability during the incubation with PHA.

Immunofluorescence Staining. After stimulation for 18 h with Ag, cell preparations were treated with 100 μl of 20 mM EDTA (final concentration, 2 mM) for 10 min to detach adherent cells, washed with cold PBS, resuspended in 1× FACS lysing solution at 5 ml/3×10^7 cells (BDIS), and left at room temperature for 10 min. Cells were washed in PBS containing 0.5% BSA and 0.1% sodium azide (buffer) and resuspended in FACS permeabilizing solution at 0.5 ml/3×10^7 cells (BDIS) for 10 min at room temperature. Cells were washed in buffer and stained for cell surface molecules and intracytoplasmic cytokines for 30 min at room temperature. After staining, cells were washed, fixed in 1% paraformaldehyde in PBS, and kept at 4°C until analyzed on the flow cytometer. Cytokines could only be detected when fresh PBMCs that had not been frozen were used (data not shown).

Flow Cytometric Analysis. Cells were analyzed on a FACScalibur flow cytometer equipped with a second 632-nm line diode laser (BDIS) using forward and side scatter parameters to identify lymphocytes, with FITC, PE, PerCP, and APC as the fluorescence markers. For each analysis, 40,000 events were usually acquired, gated on a logical gate of viable lymphocytes and CD3, CD4, or CD8 expression (most files required fine tuning after acquisition), and analyzed using the CELL QUEST program (BDIS) for $CD69^+$ cytokine-producing cells. Isotype matched antibodies were used to verify the staining specificity and as a guide for setting the markers to delineate positive and negative populations. The intra- and interassay variations were found to be <10%. The results are represented as follows: (*a*) net percentage positive after subtraction of background; or (*b*) the ratio of test Ag to the no-Ag control (Ag^+/Ag^-) in comparisons of samples stimulated with the same Ag.

RESULTS

Parameters for MUC1 T Cell Cytokine Responses. The use of the intracytoplasmic staining for cytokines has previously been demonstrated for mitogens, superantigens, and viral peptides (19, 20) but not for tumor immunotherapy. Before measuring the level of intracellular cytokines secreted by T cells after stimulation with MUC1, we examined the parameters for optimal cytokine secretion in activated $CD69^+$ cells. Cells from an immunized patient were stimulated with M-FP for a period of 18 h; within 6 h of stimulation, peak levels of activated cells were reached, at which 30.8% of the cells were activated, as shown by the presence of $CD69^+$ cells (Fig. 1*A*). Cytokine levels had increased by 6 h to reach a maximum by ~18 h. At that time TNF-α production was the highest, with 5.5% of $CD3^+CD69^+$ containing $TNF\text{-}\alpha^+$ cells *versus* 0.14% at 3 h (39-fold increase; Fig. 1*B*). $CD69^+$ $IFN\text{-}\gamma^+$ cells had increased 9-fold by 18 h with 1.7% $CD3^+CD69^+$ containing $IFN\text{-}\gamma^+$ cells *versus* 0.19% at 3 h, whereas IL-2 containing cells showed a 2-fold increase (1.4% $CD3^+CD69^+IL\text{-}2^+$ *versus* 0.68% at 3 h). Thus, for further studies, 18 h of stimulation with the MUC1 Ag was used.

BFA is used in studies to detect intracellular cytokines as it inhibits their extracellular transport; the time of exposure to BFA for the detection of TNF-α, IL-2, and IFN-γ in activated T cells was examined (Fig. 1*C*). BFA was added either 1, 2, or 3 h after the addition of M-FP and remained in the culture. When BFA was added after 1 h of culture, the ratio of $CD4^+CD69^+$ cytokine-producing cells (calculated as the number of $CD4^+CD69^+$ cytokine-producing cells stimulated with M-FP divided by the no-Ag control) was 2.5 for TNF-α, 1.2 for IL-2, and 1.2 for IFN-γ. When added after 2 h of culture, the ratio of cytokine-producing cells was 4.6 for TNF-α and 1.9 for IFN-γ but was unchanged for IL-2 (1.1). After 3 h of incubation with BFA, the ratio of cytokine-producing cells had decreased. Thus, BFA was added after 2 h of exposure to Ag, and a further 16 h of culture was performed; these did not reduce the viability of cytokine-producing cells.

Previous studies had found that the addition of the CD28 mAb enhanced the ability to detect intracellular cytokines after stimulation with Ag (24); PBMCs were therefore stimulated with M-FP in the presence and absence of the CD28 mAb (Fig. 2). The addition of the CD28 mAb increased, by 8-fold, the number of $TNF\text{-}\alpha^+$ and $IFN\text{-}\gamma^+$ cells in $CD8^+CD69^+$ T cells ($IL\text{-}2^+$ cell numbers remained low; data not shown). After stimulation of PBMCs with M-FP and CD28, several findings were apparent: (*a*) 1.05% $CD8^+CD69^+$ contained $TNF\text{-}\alpha^+$ cells *versus* 0.12% in the absence of CD28 (Fig. 2, *B* and *C*); (*b*)

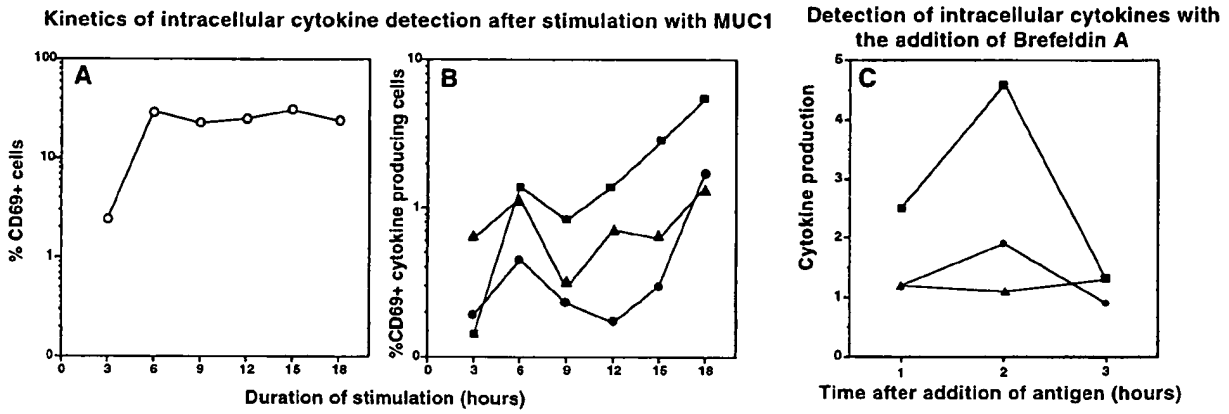

Fig. 1 Optimal parameters for the detection of intracellular cytokines after stimulation with MUC1. PBMCs were stimulated according to the panel description below, and cells were fixed, permeabilized, and stained with antibodies to CD3 and CD69 (○), TNF-α (■), IFN-γ (●), or IL2 (▲). 40,000 events gated on viable $CD3^+$ (*A* and *B*) or $CD4^+$ (*C*) lymphocytes were analyzed for $CD69^+$ cytokine-producing cells. *A*, kinetics of PBMC activation after stimulation with M-FP for the indicated time (h; *x* axis), with CD28 and M-FP (50 μg/ml); results are expressed as the $\%CD69^+$ (*y* axis). *B*, kinetics of intracellular cytokine generation after stimulation with M-FP for the indicated time (h; *x* axis) with CD28 and M-FP (50 μg/ml); results are expressed as the $\%CD69^+$ cytokine-producing cells (*y* axis). *C*, determination of optimal time of incubation with BFA. PBMCs were stimulated *in vitro* with CD28 and M-FP (50 μg/ml), BFA was added 1, 2, or 3 h (*x* axis) after the addition of Ag, and the experiment was terminated after 18 h of culture. The results are expressed as cytokine production (*y* axis) calculated as the number of $CD4^+CD69^+$ cytokine-producing cells stimulated with M-FP divided by the no-Ag control ($M\text{-}FP^+/Ag^-$).

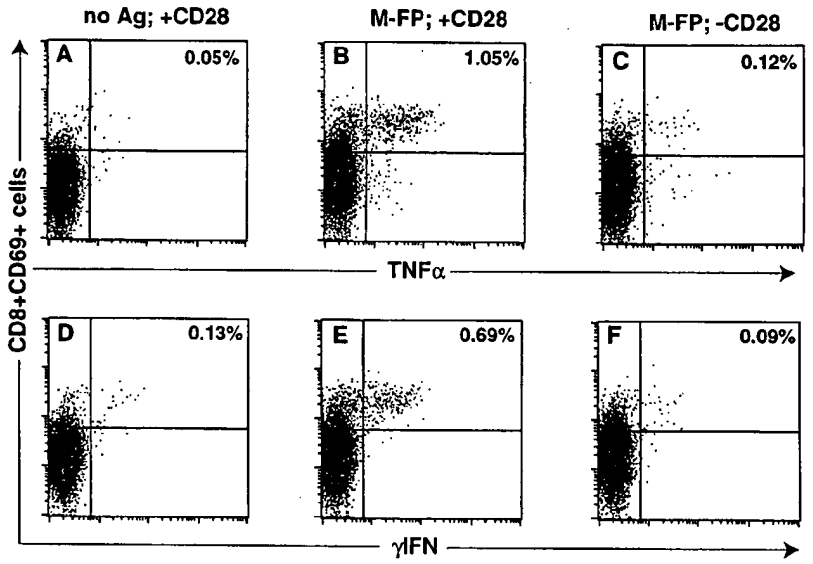

Fig. 2 Coculture with CD28 mAb and Ag enhances the levels of cytokine generation. PBMCs were stimulated *in vitro* for 18 h with either CD28 mAb and no Ag (*A* and *D*), CD28 mAb and M-FP (50 μg/ml; *B* and *E*) or M-FP (50 μg/ml) and no CD28 mAb (*C* and *F*). Cells were stained with antibodies to CD8 or CD69 (*y* axis) and TNF-α or IFN-γ (*x* axis). 40,000 events gated on viable $CD8^+$ lymphocytes were analyzed for $CD69^+$ cytokine-producing cells. The numbers in the *upper right quadrant* of each panel represent the $\%CD8^+CD69^+cytokine^+$ cells.

0.69% of $CD8^+CD69^+$ cells contained IFN-γ^+ cells in the presence of the CD28 mAb *versus* 0.09% in its absence (Fig. 2, *E* and *F*); and (*c*) the CD28 mAb alone did not generate significant cytokine production (Fig. 2, *A* and *D*). Thus, all experiments used a culture period of 18 h with Ag and CD28 mAb, with BFA being added after 2 h.

Detection of Cytokines after Stimulation with Tetanus Toxoid, Influenza Ags, or PHA. To further validate the intracytoplasmic cytokine measurements, we sought intracellular cytokines in PBMCs: (*a*) in normal subjects injected with either tetanus toxoid or influenza vaccine; or (*b*) in normal subjects and patients with adenocarcinoma after stimulation with the mitogen PHA. In a subject immunized with influenza vaccine 60 days earlier, $CD3^+$ cells were examined for intracellular cytokines before and after immunization (Fig. 3*A*). Compared with preimmune results, the ratio of $CD3^+CD69^+$ cytokine-producing cells (calculated as the number of $CD3^+CD69^+$ cytokine-producing cells stimulated with influenza divided by the no-Ag control) had increased 30 days after immunization and had returned to the preimmune level by 60 days. TNF-α accumulation was the greatest, with the ratio being 68.0 (day 30) *versus* 1.9 (preimmune day 0) and 3.4 (day 60). IFN-γ showed ratios of

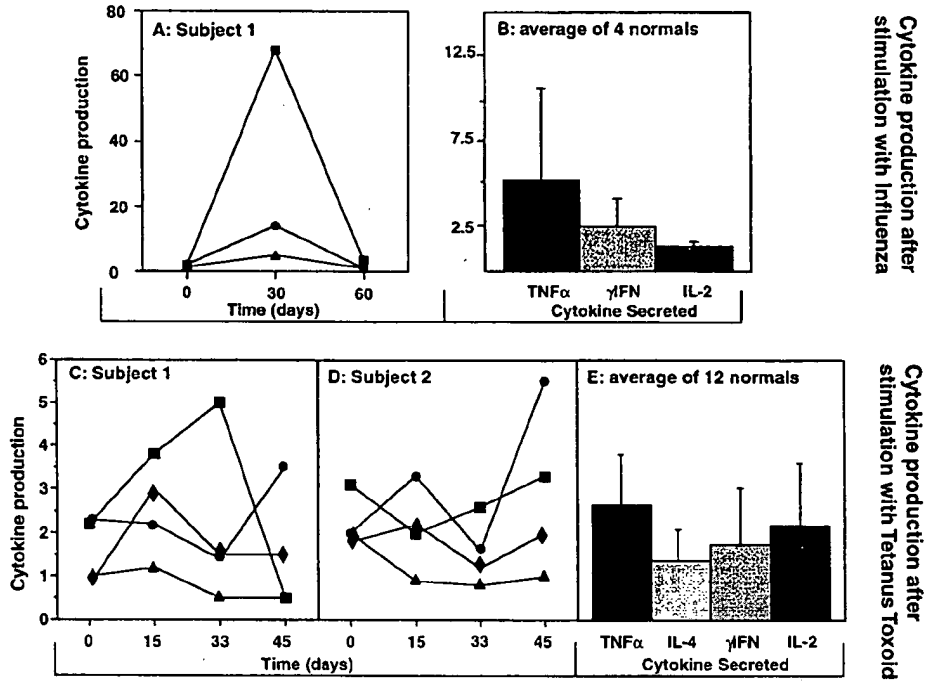

Fig. 3 Intracellular cytokine production after stimulation with recall Ags. PBMCs from normal subjects were stimulated for 18 h *in vitro* with influenza vaccine (10 μg/ml) or tetanus toxoid (10 μg/ml) and CD28 mAb. Cells were fixed, permeabilized, and stained with antibodies to CD3 or CD4, CD69 and TNF-α (■), IFN-γ (●), IL-2 (▲), or IL-4 (♦). Forty thousand events gated on viable $CD3^+$ (*A* and *B*) or $CD4^+$ (*C–E*) lymphocytes were analyzed for $CD69^+$ cytokine-producing cells. The results are expressed as cytokine production (*y* axis) calculated as the number of $CD69^+$ cytokine-producing cells stimulated with either influenza vaccine or tetanus toxoid divided by the no-Ag control (Ag^+/Ag^-). *A,* serial PBMC samples from a normal subject vaccinated recently with influenza vaccine (*x* axis: time after immunization at day 0). *B,* PBMCs from four normal subjects who had not been deliberately immunized but had presumably been exposed to the influenza virus the previous year. *C* and *D,* serial PBMC samples from two normal subjects immunized recently with tetanus toxoid (*x* axis: time after immunization at day 0). *E,* PBMCs from 12 normal subjects who received tetanus toxoid immunization within the last 3 years.

2.1 (day 0), 14.0 (day 30), and 1.0 (day 60); IL-2 ratios were 1.4 (day 0), 5.0 (day 30), and 1.0 (day 60). At day 30, all three cytokines were present at a higher level than was found in four normal subjects who had presumably been exposed to the influenza virus the previous year (Fig. 3*B*). In two subjects who received tetanus toxoid booster injections, the cytokines generated by CD4 cells were examined before and after immunization (Fig. 3, *C* and *D*). In subject 1, the ratio of $CD4^+CD69^+$ cytokine-producing cells (calculated as the number of $CD4^+CD69^+$ cytokine-producing cells stimulated with tetanus toxoid divided by the no-Ag control) for TNF-α was 2.2 before immunization and 5.0 at 33 days after immunization. This was higher than the mean of 12 normal subjects who were immunized >2 years prior to testing (mean ratio, 2.6 ± 1.2; Fig. 3*E*). The number of IFN-γ^+ cells did not rise significantly, but at 45 days after immunization, the ratio was higher than in 12 normal subjects (mean ratio, 1.3 ± 0.8). IL-2 and IL-4 measurements were not different from that of the normal subjects. In $CD4^+CD69^+$ cells from subject 2, TNF-α did not alter, IFN-γ was higher than normal at 45 days, and, again, IL-2 and IL-4 were not different from the normal subjects. Thus, subjects immunized with influenza or tetanus toxoid showed measurable increases in the intracellular cytokines TNF-α or IFN-γ but not IL-2 and IL-4 in $CD4^+$ cells; similar findings occurred with $CD8^+CD69^+$ cells.

To measure the ability of PBMCs from cancer patients immunized with M-FP and nonimmunized individuals to respond to a T cell mitogen, cells were stimulated for 18 h with PHA and $CD69^+$ cells producing TNF-α, IL-4, or IFN-γ, which was measured (Fig. 4). Both $CD4^+CD69^+$ and $CD8^+CD69^+$ T cells from patients and normal subjects produced TNF-α. However, the ratio of cytokine-producing cells (calculated as the number of $CD4^+CD69^+$ or $CD8^+CD69^+$ cytokine-producing cells stimulated with PHA divided by the no-Ag control) was higher in the $CD8^+$ cells of patients and the $CD4^+$ cells of normal subjects (Fig. 4*A*). Furthermore, IL-4 production was the same for patients and normal subjects (Fig. 4*B*), whereas IFN-γ production was higher in $CD8^+CD69^+$ T cells of patients and normal subjects (Fig. 4*C*). Thus, cells from cancer patients were able to respond to mitogens by producing cytokines; it was therefore appropriate to examine their responses after MUC1 immunization.

Cytokine Responses to MUC1 in Several Subjects. Intracellular cytokine production in response to a MUC1 stimulus was examined in $CD4^+CD69^+$ and $CD8^+CD69^+$ cells from patients with adenocarcinoma who had been immunized with mannan MUC1. For these experiments, PBMCs were activated in autologous plasma with M-FP or VNTR and stained with CD3 APC-, CD8 PerCP-, CD69 PE-, and FITC-conjugated cytokine mAbs (to TNF-α, IL-4, and IFN-γ). Prior to examining

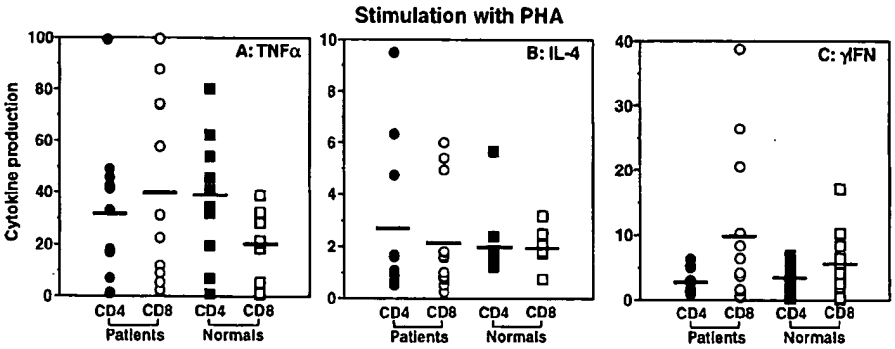

Fig. 4 Mitogen stimulation to induce intracellular cytokine. PBMCs from 12 patients with adenocarcinoma immunized with MUC1 (● and ○) and 11 normal subjects (■ and □) were stimulated with PHA. Cells were fixed, permeabilized and stained with antibodies against CD4, CD8, CD69, and TNF-α (*A*), IL-4 (*B*), and IFN-γ (*C*). Forty thousand events gated on viable $CD4^+$ (● and ■) or $CD8^+$ (○ and □) lymphocytes were analyzed for $CD69^+$ cytokine-producing cells. The results are expressed as cytokine production (*y* axis), calculated as the number of $CD69^+$ cytokine-producing cells stimulated with PHA divided by the no-Ag control (PHA^+/Ag^-). The mean for each group is shown (*horizontal line*).

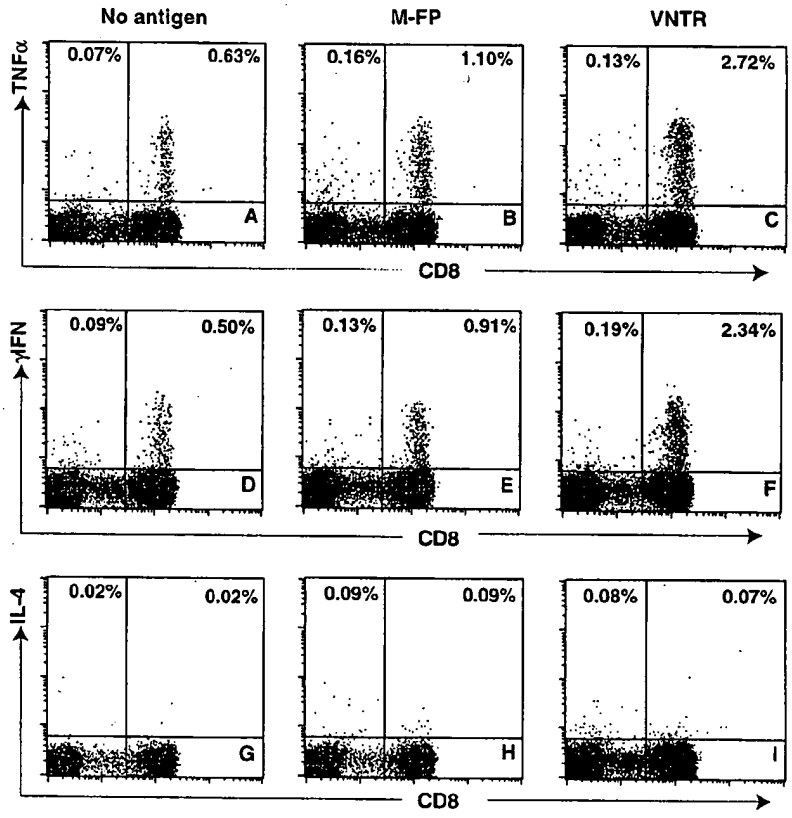

Fig. 5 Cytokine profile of a patient immunized with M-FP. PBMCs were stimulated *in vitro* for 18 h either with no Ag (*A, D,* and *G*), M-FP (50 μg/ml; *B, E,* and *H*), or VNTR (20 μg/ml; *C, F,* and *I*). Cells were stained with antibodies to CD3, CD8, and TNF-α (*A–C*), IFN-γ (*D–F*), or IL-4 (*G–I*). Forty thousand events gated on viable $CD3^+$ lymphocytes were analyzed for the presence of cytokines. The percentage of $cytokine^+$ cells is indicated in each plot ($CD8^+$ in the *right quadrant* and $CD4^+$ in the *left quadrant*). The number of $CD4^+$ cells (CD8-negative population; *top left*) staining for cytokines was calculated after subtracting the percentage contributed by the $CD8^{low}$ cells.

the 12 patients, several subjects were examined to determine which cytokines were the most appropriate and what was the best time to examine patients cells after immunization. Fig. 5 shows representative three-color plots of CD8 T cells from a patient after four M-FP immunizations, whose PBMCs were cultured without Ag (Fig. 5, *A, D,* and *G*), with M-FP (*B, E,* and *H*), or with VNTR (*C, F,* and *I*). In general, better responses were seen with MUC1 VNTR than with M-FP. Thus, for TNF-α, the reactive cells after VNTR stimulation were 2.72% *versus* 1.10% after M-FP (no Ag, 0.63%) and for IFN-γ, 2.34% after VNTR stimulation *versus* 0.91% after M-FP (no Ag, 0.50%) *i.e.*, VNTR gave a greater than 2-fold increase in the number of cells detected. Furthermore, the cytokine responses seen in $CD8^+CD69^+$ cells were greater than those of $CD4^+CD69^+$ cells, as the number of $TNF\text{-}\alpha^+$ and $IFN\text{-}\gamma^+$ cells after stimulation with M-FP and VNTR was similar to that after

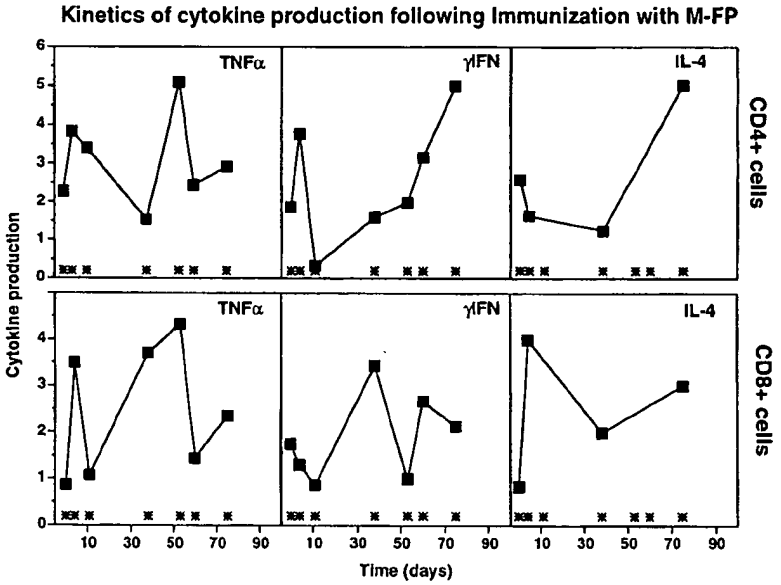

Fig. 6 MUC1-specific cytokine responses increase with multiple M-FP immunizations. Blood was obtained from a patient with adenocarcinoma immunized with MUC1 at various time points (*x* axis). PBMCs were stimulated with M-FP, and cells were stained with antibodies to CD4, CD8, CD69, and TNF-α, IFN-γ, or anti-IL4. Forty thousand events gated on viable $CD4^+$ or $CD8^+$ lymphocytes were analyzed for $CD69^+$ cytokine-producing cells. The results are expressed as cytokine production (*y* axis) calculated as the number of $CD69^+$ cytokine-producing cells stimulated with M-FP divided by the no-Ag control ($M\text{-}FP^+/Ag^-$). The times of testing (■) and vaccination (*) are shown.

stimulation with no Ag (note that in Fig. 5, the $CD4^+$ cells were calculated by subtracting the $CD8^+$ fraction from $CD3^+$). Finally, neither $CD8^+CD69^+$ nor $CD4^+CD69^+$ cells produced significant amounts of IL-4. Thus, subsequent measurements concentrated on IFN-γ, TNF-α, and IL-4 in $CD4^+CD69^+$ and $CD8^+CD69^+$ cells.

Most of the samples from immunized patients were tested 1–2 weeks after their last immunization with M-FP (in 2 patients, the samples were tested 25 weeks after the last immunization). Previously, using a CTL response, we were unable to identify the optimal time needed between immunization and testing (25). However, because of the small volume of blood needed to perform flow cytometric analysis, the intracellular cytokine production could be examined soon after M-FP immunization (Fig. 6). Serial PBMC samples collected from a patient receiving immunizations with M-FP were stimulated with M-FP and $CD4^+CD69^+$ and $CD8^+CD69^+$ cells were examined for the presence of TNF-α-, IL-4-, and IFN-γ-producing cells. To minimize possible errors in our calculations of cytokine-producing cells, we included in each test a PHA-positive control, which indicated a <10% interassay variability (data not shown). It was of interest that $CD8^+CD69^+$ T cells could be shown to have increases in TNF-α and IFN-γ after four injections, whereas in this study, IL-4 increased after the first injection. Furthermore, $CD4^+CD69^+$ T cells demonstrated an increase IFN-γ-producing cells after the third immunization, and so responses were measured 7 days after the fourth immunization.

Cytokine Responses in 12 Immunized Subjects. The intracellular cytokines present in $CD4^+CD69^+$ and $CD8^+CD69^+$ T cells were then examined in 12 immunized and 10 normal subjects (Fig. 7). The samples from immunized patients were tested 1–2 weeks after their last of seven immunizations with M-FP. The ratios of cytokine-producing cells (calculated as the number of $CD4^+CD69^+$ or $CD8^+CD69^+$ cytokine-producing cells stimulated with M-FP or VNTR divided by the no-Ag control) after stimulation with M-FP (Fig. 7, *A–C*) or VNTR (Fig. 7, *D–F*) were determined. Several findings were apparent: (*a*) the responses of all cytokines to VNTR peptide were greater than (M-FP); (*b*) $CD8^+CD69^+$ T cells from MUC1-immunized subjects contained more cells expressing TNF-α after VNTR stimulation than those from nonimmune subjects (2.6 *versus* 0.76; $P < 0.038$); (*c*) the same finding occurred with $CD8^+CD69^+$ cells and IFN-γ (4.5 *versus* 0.4; $P < 0.01$); (*d*) although IL-4 also increased in $CD8^+CD69^+$ cells (2.0 *versus* 0.8), this was not significant; (*e*) $CD4^+CD69^+$ cells stimulated with either M-FP or VNTR showed no significant differences between immunized and normal subjects for TNF-α, IFN-γ, or IL-4, although individual patients exhibited some alterations.

Thus, the responses in 12 immunized patients were statistically significant for TNF-α and IFN-γ when the VNTR peptide was used and $CD8^+CD69^+$ cells were examined; in these cells, IL-2 (not shown) and IL-4 were not altered. There were no differences when $CD4^+CD69^+$ cells were examined for the cytokines IL-2, IL-4, IFN-γ, and TNF-α.

DISCUSSION

Immunotherapy has potential for the treatment of some forms of cancer, in which patients are immunized with an immunogen/adjuvant combination and the effectiveness of the immunization is measured as the amplification or generation of immune responses (25, 26), as reproducible tumor responses have not been found as yet. For solid tumors it is likely that $CD8^+$ cytotoxic T cells are those required for tumor elimination—acting by cytotoxicity and/or cytokine release—particularly the T1 cytokines IL-2, IFN-γ, and TNF-α. However, the *in vitro* measurement of CTL responses in patients with cancer is difficult: compared to murine studies, in which the spleen or lymph nodes are used, in humans, peripheral blood must be

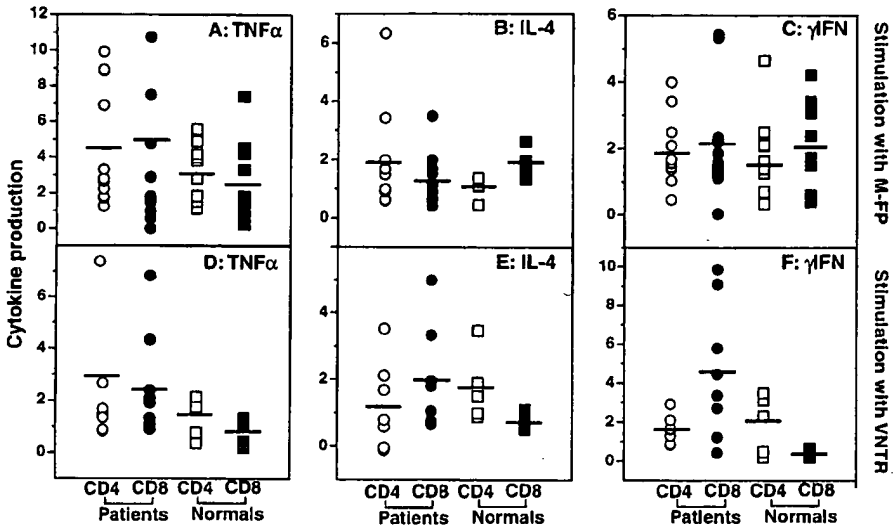

Fig. 7 Intracellular cytokines in MUC1-immunized patients. Intracellular cytokines were detected from 12 patients with adenocarcinoma immunized with MUC1 (○ and ●) and 11 nonimmunized normal subjects (□ and ■). PBMCs were stimulated for 18 h with M-FP (*A–C*) and VNTR (*D–F*). Cells were stained with antibodies to CD4, CD8, CD69, and TNF-α, IL-4, and IFN-γ. Forty thousand events gated on viable $CD4^+$ (○ and □) or $CD8^+$ (● and ■) lymphocytes were analyzed for $CD69^+$ cytokine-producing cells. The results are expressed as cytokine production (*y* axis) calculated as the number of $CD69^+$ cytokine-producing cells stimulated with Ag divided by the no-Ag control (Ag^+/Ag^-). The mean for each group is shown (*horizontal line*).

taken, and although there may be CTLs infiltrating the tumors, often few are in the peripheral blood. To overcome these difficulties, multiple rounds of restimulation *in vitro* are used, and it is questionable how such CTL results relate to what was originally present in the patients. Furthermore, it is difficult to immunize patients with cancer (often with very advanced disease in Phase I studies), and there are currently searches for new modes of measuring T cell activation and activity in patients with cancer (27). We now report that in patients immunized with mannan MUC1, there are measurable numbers of $CD4^+$ and $CD8^+$ activated $CD69^+$ T cells producing intracellular cytokines; the studies were performed on freshly collected PBMCs stimulated for 18 h with Ag.

The results were clear: (*a*) $CD8^+CD69^+$ T cells from immunized patients produced, intracellularly, the T1 cytokines IFN-γ and TNF-α; (*b*) $CD4^+CD69^+$ cells showed the same trend, with IFN-γ and TNF-α, but the increases were not significant: (*c*) there was a modest increase in IL-4 production but not IL-2 production [neither one was produced in significant amounts in the 12 patients; clearly, the major responding ($CD69^+$) cells producing cytokines were $CD8^+$ T cells]. Optimal results were obtained in the presence of a CD28 mAb and required Brefeldin to be added after 2 h of culture with the VNTR peptide but not with the MUC1 fusion protein. On the basis of these results, we can state that detection of intracellular cytokines is a simple and reliable method of measuring T cell responses in patients with cancer. Thus, the detection of immune responses in the cells of the 12 MUC1-immunized patients required three conditions: the appropriate cells ($CD8^+$ and $CD69^+$, not $CD4^+$ and $CD69^+$), Ag in the correct form (VNTR but not M-FP), and measurement of the appropriate cytokines (IFN-γ and TNF-α but not IL-2, IL-4, or IL-8). The results in the 12 patients convincingly demonstrated that $CD8^+$ cells were making T1 cytokines, and the method was substantially simpler to perform than the earlier CTL/CTLp and T cell proliferation studies and delayed hypersensitivity testing (25).

There are a number of technical aspects that require further comment. First, in these patients, the detected cytokines did not survive freezing and thawing of the cells; thus, tests have to be performed on the day the patient's blood is taken, but the results are then available within 24 h. It may be desirable to perform all tests simultaneously on cells taken over the period of immunization; to avoid variations in responses, however, the variations that we found in Ag responses in normal individuals were small, and we consider this not to be a major problem. There are real advantages in having the answers within 24 h: during this time ELISA tests are done to measure antibody, and an assessment of the immune status can be rapidly determined. A second technical aspect was that the use of Brefeldin (addition after 2 h of Ag stimulation) and the culture period (culturing for a further 16 h) are different from studies in viral diseases (19, 20, 24). This is not surprising and indicates that in each disease and possibly for each peptide or antigenic system, the appropriate time of culture with Brefeldin has to be assessed. It was also apparent that improved responses were obtained in the presence of the CD28 antibody, in agreement with other studies (24). A third aspect to consider is which cytokines to measure. Initially, we examined IL-2, IL-4, IL-8, IFN-γ, and TNF-α, but as the study progressed, IL-8 was abandoned, as it was present nonspecifically in some of the patients; because it is produced by natural killer cells and neutrophils rather than T cells (28, 29), it was not examined further. Measurements of IL-2 or IL-4 were not useful measurements, which is of interest. As described previously (25), patients make a significant MUC1 antibody response to the immunizing peptide, with titers in excess of 1:10,000 by ELISA. These are presumably due to a T2 type response from $CD4^+$ cells, and therefore, IL-4 was expected to be present, but CD4 cells making IL-4 could not be detected. Perhaps the peripheral blood is not the place to seek cytokine-producing cells for antibody responses, and a different profile may well have been present in lymph nodes or spleen.

Our findings of $CD8^+IFN\text{-}\gamma^+$ and $CD8^+TNF\text{-}\alpha^+$ cells indicate a phenotypic pattern suggestive of a T1 response in patients vaccinated with MUC1. Particularly after stimulation

with the VNTR peptide, CD8 T cells from immunized patients produced 9 times more IFN-γ-producing cells than did CD8 T cells from normal patients (30, 31); however, we note that to this extent, $CD45RO^{high}$ memory effector cells have such a cytokine profile (32, 33). The immunization using mannan MUC1 gives T1 responses in mice, with a cytotoxic T cell response, little antibody, and IFN-γ, TNF-α, and IL-12 secretion (4). However, in our patients, although CTLs were found in approximately ~20% (25), more patients (~60%) made antibodies—possibly a T2 response due to the cross-reaction of MUC1 peptides with antigalactosidase antibodies (34), leading to immune complex formation. Moreover, the cellular responses measured by the flow cytometric analysis of intracytoplasmic cytokines were of higher frequency than found previously, and so the measurement of cytokines may well be a more sensitive assay to measure MUC1 cellular responses (25). However, these were different patients, and unfortunately, we were not able to measure both CTLs and intracytoplasmic cytokines in the same set of patients, although it should not be surprising that both T1 and T2 responses can occur in the same patient, given the complexity of the Ag administered. [The Ag consists of a 100-mer linked to mannan, which is known to contain epitopes that can be presented by both Class I molecules (epitopes have been mapped for both murine and human MHC Class I molecules) and must also contain Class II presenting molecules (which have not been mapped, but the peptide gives rise to T-dependent high antibody responses)]. In addition, we had shown previously that the administration of oxidized and reduced mannan MUC1 together could give rise to both T1 and T2 responses (35); perhaps the same occurs in patients.

Thus, the measurement of intracytoplasmic cytokines is simpler and gives a higher frequency than the measurement of CTLs, and as the numbers are higher, one would be more inclined to accept these results than those from CTLs. However, these results highlight one of the major difficulties with cancer therapy: there is no clinical response, such as tumor shrinkage, to help decide what is the optimal type of immune response and how it should be measured. Thus, the ideal situation would be to have tumors disappearing and relate this to defined cellular assays (be they tetramer binding, intracytoplasmic cytokines, CTLs, or Elispot or cytokine secretion in plasma); only in this way can the meaning of the results of the different tests be determined.

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Detection of Intracellular Antigen-Specific Cytokines in Human T Cell Populations

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Determination of antigen-specific cytokine responses of T lymphocytes after vaccination is made difficult by the low frequency of responder cells. In order to detect these responses, the profile of intracellular cytokines was analyzed using flow cytometry after antigenic expansion. Peripheral blood mononuclear cells were stimulated with antigens for 5 days, further expanded with interleukin (IL)-2, and then restimulated on day 10. Cytokine production was detected by intracellular staining with monoclonal antibodies after saponin-based permeabilization. Influenza expansion resulted in specific interferon-γ (IFN-γ) production of 6%–20%, with less IL-4 production (0%–2%). Tetanus toxoid resulted in even greater production. IL-4 and IFN-γ were produced mainly by memory cells of the CD45RO$^+$ phenotype. IFN-γ production was contributed by both CD4 and CD8 populations. These methods were then applied to a clinical trial of a candidate human immunodeficiency virus type 1 vaccine. Antigen-specific increases in IFN-γ were measured, which corresponded to antibody production, lymphoproliferation, and skin testing.

Cellular and humoral responses to vaccinations or to infections recently have been characterized by the cytokine production of activated peripheral blood lymphocytes or monocytes [1–3]. Profiles of cytokine production after infection or vaccination in animal models are often interpreted by a TH1/TH2 paradigm in which protection can be associated with either response [4–8]. In general, the TH1 profile is characterized by production of antigen-specific interferon (IFN)-γ, interleukin (IL)-12, and complement-fixing antibodies, while the TH2 phenotype is characterized by production of IL-4, -5, and -10 and an increase in IgE and isotype switching [9, 10]. This cytokine production by human peripheral blood mononuclear cells (PBMC) or murine lymph node lymphocytes can be measured directly by intracellular protein staining [11–13]. This technique allows the analysis of cytokine production at the single cell level and the determination of whether cells are pluripotent for production of more than one cytokine. In almost all studies performed to date, these measurements have been performed after mitogenic stimulation and therefore reflect the general, global potential of the PBMC. In settings of profound ongoing antigenic stimulation, such as human immunodeficiency virus type 1 (HIV-1) infection, schistosomiasis, or cytomegalovirus (CMV) viremia, quantification of the TH1/TH2 effector response may be possible by antigenic or mitogenic stimulation of the circulating effector PBMC. However, after most immunizations, the number of circulating antigen-specific effector cells is relatively small, and the low frequency of the antigen-specific T cell memory response may be lost in the background signal when the bulk response to mitogen is measured.

Although recent studies have shown the ability to detect circulating CD8-specific cytokines with minimal stimulation when the major histocompatibility complex class I epitope is known [14], no direct method for the detection of cytokines in PBMC after vaccination has been published. An example of the difficulty in measuring TH1/TH2 responses after disease or vaccination is the failure to detect antigen-specific IL-4 after vaccination, even when large amounts of antibody are produced. IL-4 has not been detected in human PBMC after brief antigenic stimulation, even in situations in which animal models would predict large amounts, such as in human visceral leishmaniasis [15]. In fact, the majority of antigen-specific TH2 responses can be measured in human PBMC only by using restimulation strategies that allow for expansion of the memory T cell population [16–18]. We and others have shown that the TH1/TH2 responses measured by these restimulation techniques have correlated with clinical responses to both immunotherapy and immune intervention [19–21]. For these reasons, we combined the methods of antigen expansion, restimulation, and intracellular staining to measure antigen-specific PBMC cytokine responses by flow cytometry in the setting of a phase 1 vaccine trial.

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Materials and Methods

Volunteers. PBMC were obtained from healthy young adults enrolled in a randomized, double-blind, placebo-controlled phase I trial of a trivalent intranasal cold-adapted, live attenuated influenza virus vaccine (Aviron, Mountain View, CA). PBMC were also obtained from participants enrolled in a double-blind phase I trial (AVEG 016B) of recombinant MN gp120 (rsgp120; VaxGen, South San Francisco), combined with either 600 μg of alum or 100 μg of the novel adjuvant QS21 (Aquila, Worcester, MA). The trial design was based on the hypothesis that the QS21 group would have a more rapid and greater antibody response than the alum adjuvant group. Twenty volunteers received 3 μg and 10 received 30 μg of rsgp120 intramuscularly on a 0-, 1-, and 6-month schedule. Half of each group received the immunization in either alum or QS21. Six volunteers received adjuvant alone. Only the participants from the University of Rochester and selected volunteers from St. Louis University were included in the cytokine studies. PBMC were obtained on day 0, 14 days after the first immunization, and 14 days after the second immunization. Skin testing was performed at 12 months, using a purified gp160 protein (MicroGeneSys, Meriden, CT) without adjuvant and with candida, mumps, and tetanus toxoid as controls.

Monoclonal antibodies (MAbs). CD45RO-fluorescein isothiocyanate (FITC) (clone UCHL1) and CD69-FITC (clone FN50), both from PharMingen (San Diego), and CD4-FITC (clone SK3), CD8-peridin chlorophyll protein (PerCP) (clone SK1), and CD3-PerCP (clone SK7), from Becton Dickinson Immunocytometry Systems (San Jose, CA), were used for cell-surface staining. Intracellular staining for cytokines was performed using phycoerythrin (PE) mouse anti–human IL-4 ($IgG_1\kappa$, clone 8D4-8) or anti–human IFN-γ ($IgG_1\kappa$, 4S.B3) (PharMingen). Isotype controls used the irrelevant IgG_1 monoclonal MOPC-21 conjugated to PE, FITC, and PerCP.

Cell preparation and stimulation. PBMC were isolated from volunteers by density-gradient sedimentation over Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). The isolated PBMC were then washed three times in Dulbecco's PBS (Gibco, Grand Island, NY). Cells were cryopreserved in RPMI 1640 (Gibco) supplemented with 20% heat-inactivated fetal bovine serum (FBS; HyClone Laboratories, Logan, UT), 50 U/mL penicillin (Gibco), 50 μg/mL streptomycin (Gibco), 10 m*M* glutamine (Gibco), and 7.5% DMSO (Sigma, St. Louis). Cryopreserved cells were stored in liquid nitrogen until used in the assays. At the time of the assay, PBMC were rapidly thawed in a 37°C water bath and washed in RPMI 1640 supplemented with 20% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 20 m*M* glutamine. Cells were counted, checked for viability, and resuspended in serum-free AIM-V (Gibco) at 10^6 cells/mL. PBMC were cultured in 24-well plates (1 mL/well) (Costar, Cambridge, MA) with the following antigens in different wells: medium (control), 100 μL of 5×10^7 pfu of heat-inactivated influenza virus (strain A/Texas H1N1) or a 5-Lf dose of tetanus toxoid (Connaught Laboratories, Toronto, Canada) at 37°C and 5% CO_2. In the AVEG 016B trial, recombinant baculovirus–produced gp160 was used at 10 μg/mL. After 5 days in culture, 500 μL of culture supernatant was removed and frozen at −70° and replaced with 500 μL of AIM-V supplemented with 40 U/mL IL-2 (Genzyme, Cambridge, MA). Cells were incubated for 5 additional days. On day 10, the contents of each well were transferred to 5-mL sterile tubes and supplemented with 1 mL of AIM-V. Cells were then stimulated with 1 μ*M* ionomycin (Sigma) and 20 ng/mL phorbol 12-myristate (PMA) (Sigma), in the presence of 2 μ*M* monensin (Sigma), for 5 h.

Control of lymphocyte phenotype and stimulation. The lymphocyte phenotype and degree of stimulation were assessed by experiments with surface staining using CD69-FITC and CD3-PerCP. Control experiments without stimulation with ionomycin and PMA were conducted on 6 samples.

Surface and intracellular staining with MAbs. After stimulation, cells were centrifuged at 1000 rpm for 15 min; an aliquot of the cell-free supernatant was saved and frozen at −70°C. The cells were resuspended in 100 μL of staining buffer (PBS supplemented with 0.1% sodium azide [Sigma] and 1% FBS, pH 7.4–7.6) and the MAbs CD45RO-FITC and CD8-PerCP and were incubated at 4°C in darkness for 15 min. After being stained, cells were washed with 2 mL of staining buffer and resuspended in 1 mL of fixation buffer (4% paraformaldehyde [Polysciences, Warrington, PA] in PBS at pH 7.4–7.6). Cells were fixed for 30 min at 4°C in darkness, centrifuged at 1000 rpm for 15 min, and resuspended in 3 mL of staining buffer. Each tube was split into 3 aliquots and centrifuged at 1000 rpm for 15 min. The cells were incubated for 30 min at 4°C in the dark with either no antibody (unstained tube), anti–IL-4–PE, or anti–IFN-γ–PE in the presence of 50 μL of permeabilization buffer (PBS supplemented with 0.1% sodium azide, 1% FBS, and 0.1% saponin [Sigma]). Cells were washed with 2 mL of permeabilization buffer and resuspended in 300 μL of staining buffer for flow cytometric analysis.

To evaluate the specificity of anti–cytokine antibody stains, blocking experiments were performed. Stimulated PBMC were surface-stained and incubated with a 5-fold excess concentration of unlabeled purified mouse anti–human IL-4 or IFN-γ in the presence of permeabilization buffer, followed by incubation with the PE-labeled mouse anti–human IL-4 or IFN-γ. Surface staining by the anti–cytokine antibody was assessed by staining the cells 5 h after PMA and ionomycin stimulation using no permeabilization.

Flow cytometric analysis. Cell samples were analyzed on a FACScan (Becton Dickinson Immunocytometry Systems). Acquisition and analysis was performed using CellQuest software (Becton Dickinson Immunocytometry Systems). Fluorescence voltages and compensation values were determined using cells (from the same blood sample of a healthy volunteer) single-stained with CD45RO-FITC or CD8-PerCP and triple-stained with CD45RO-FITC, anti–IFN-γ–PE, and CD8-PerCP. For each tube, 10,000 events were acquired in a stored live lymphocyte gate.

To analyze the results, a template was set up for each sample collected, which contained the plots and histograms of each specific antigen. A gate on the lymphocytes was drawn using the side- and forward-scatter plot. The quadrant markers used to determine the percentage of lymphocytes producing IL-4 or IFN-γ were set from the unstained tube dot plot of the unstained sample. There was no difference between isotype controls and unstained samples in setting gates. The percent antigen-specific cytokine response was controlled for by subtracting the medium-stimulated cytokine, based on the following formula (figures 2–4): [(antigen-expanded % anti–cytokine-stained) − (antigen-expanded unstained control)] − [(me-

dium-expanded % anti–cytokine-stained) − (medium-expanded unstained control)].

Control of CD8 proportion during the experiments. To address the variability in stimulation and staining on different days, a blood sample from a healthy unvaccinated volunteer was obtained; PBMC were separated as described above and cryopreserved in 9 aliquots. On the day of each experiment, 1 aliquot was thawed, and a control and tetanus toxoid stimulation were set up in parallel with the cryopreserved PBMC from the participants of the study. At day 10, the control cells were subjected to the same surface staining, fixing, and intracellular staining steps as the samples from the volunteers.

Statistical analysis. The pre- and postvaccination cytokine responses were compared by analyzing the gp160-induced INF-γ by a Wilcoxon matched pairs test using Statistica/w software (version 5.1E; StatSoft, Tulsa, OK). Tetanus responses were used as an internal control for each volunteer.

Results

Mitogens enhanced cytokine production in vitro. In repeated experiments, the 5-h mitogenic stimulation of freshly isolated PBMC with the combination of PMA (3–40 ng/mL) and a calcium ionophore (1 μM) in the presence of monensin (2 μM) resulted in percentages of total cells stained intracellularly for IFN-γ (4%–20%) and IL-4 (0%–3%) similar to values previously reported [11–13]. With the use of directly conjugated PE-labeled anti–cytokine antibodies, little background staining was found. Attempts to lower the background staining (0.1%–0.6% for IFN-γ) in nonstimulated cells with either dry milk or excess irrelevant isotype antibody did not improve on the background staining resulting from direct staining alone (data not shown).

After long-term culture of PBMC, CD4 and CD8 can be separated by $CD8^+$ gating. When the cells were further surface-stained with either CD4 or CD8, along with CD45RO, the relative contribution of these two subsets could be delineated. Because the use of PMA and ionomycin resulted in a much larger down-regulation of CD4 than of CD8, we chose to use the CD8 as a marker in our experiments. In three separate experiments, the percentage of cells that were IL-4–positive and CD4- (down-regulated)–positive was similar to the percentage of cells that were CD8 negative (which is down-regulated to a lesser extent by the mitogen stimulation) and IL-4–positive. Similar results were found for IFN-γ. We therefore chose to use CD8, CD45RO as the standard surface-staining procedure in the cytokine experiments.

Long-term antigen stimulation of lymphocytes with mitogen restimulation elicits cytokine expression. We performed initial studies in an attempt to delineate antigen-specific responses without mitogenic restimulation. PBMC were cultured with tetanus toxoid, and IL-2 was added at 5 days to promote the further expansion of the relevant population. When the cells were cultured for 7–12 days and monensin was added for the last 6–8 h of growth, we were unable to detect antigen-specific IFN-γ responses. Since an antigen expansion with mitogen restimulation strategy [16] was successful for detecting soluble cytokine production by ELISA [21], this strategy was also adapted for flow methodology. Cells were expanded for 10 days in the presence of antigen, and IL-2 was added for the last 5 days. They were then washed, mitogenically restimulated with PMA and ionomycin in the presence of monensin, surfaced-stained, fixed, and intracellularly stained.

High-level cytokine production was then easily detected in mitogen-restimulated cells. Parallel experiments in PBMC after 10 days of exposure in vitro to either tetanus toxoid or influenza antigen revealed a significant increase in the percentage of both CD4 and CD8 cells expressing IFN-γ when stimulated versus unstimulated cells were compared (figure 1). There was a small, but nonstatistically significant, difference in the percentage of cells staining for IL-4 after initial stimulation with tetanus or influenza followed by restimulation with medium or PMA and ionomycin (data not shown). However, in experiments using either herpes simplex virus or CMV antigen we were easily able to measure antigen-specific IL-4 by flow cytometry, which was confirmed at the mRNA level [22]. In these experiments, no specific IFN-γ production was measured in herpes simplex virus– or CMV-seronegative persons.

PBMC were also frozen in 9 aliquots from a single donor, and 1 sample was thawed for each experimental set-up during the influenza trial. This allowed monitoring of the variation in culture techniques or in stimulation intensity from experiment to experiment. To control for the number of contaminating B

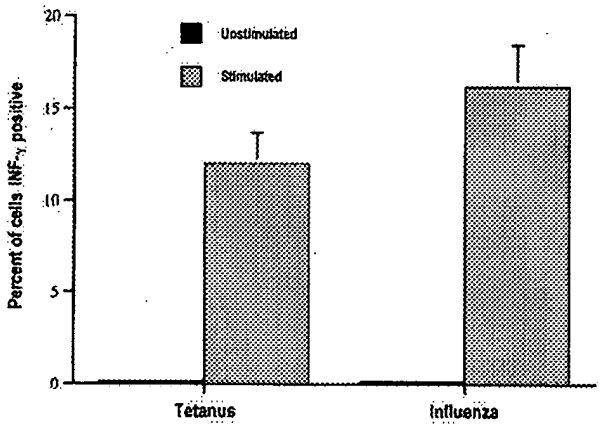

Figure 1. Peripheral blood mononuclear cells from healthy controls (n = 22) were cultured in AIM-V for 10 days with medium alone, influenza antigen, or tetanus toxoid (as per Materials and Methods) and then stimulated with either medium control or phorbol myristate acetate (PMA) and ionomycin in presence of 2 μM monensin for 6 h. Cells were then surface-stained for CD8 and intracellularly stained. Graphic shows % interferon-γ production in $CD8^-$ population for either unstimulated or PMA and ionomycin-stimulated cells. For both tetanus and influenza, medium control (either unstimulated or PMA- and ionomycin-stimulated) has been subtracted from same sample.

cells, NK cells, or monocytes in the lymphocyte gate, the CD3 population percent was assessed in the 10-day cultures. In four separate experiments, the nongated, nonadherent cells comprised at least 92% $CD3^+$ cells. Therefore, the $CD8^-$ population is essentially $CD4^+$ T cells (after a lymphocyte gate is applied). The percentage of $CD8^+$ and $CD8^-$ cells in the 10-day cultures remained replicable in eight separate experiments on aliquoted cells from the single donor ($CD8^+$ percent: mean = 38.6, SE = ±1.9).

The extent of mitogen stimulation after antigen expansion was assessed by examining the expression of CD69 in the stimulated versus nonstimulated population. Essentially all of the stimulated cells expressed this activation marker after 6 h. Thus, the percentage of cytokine-producing cells after 10 days of culture in these experiments represents the true overall potential for cytokine production in this expanded cell population.

Detection of cytokine production in naive and memory subsets. These techniques were then used for volunteers who were enrolling in an influenza vaccine trial. As can be seen in figure 2, antigen-specific expansion to all of the antigens was found. The number of IFN-γ–positive cells in the medium control that were restimulated with PMA and ionomycin ranged from 0% to 10% and averaged ~1%–2%. These values were subtracted from those for the antigen-stimulated cells for each sample. The percentage of cells expressing IFN-γ ranged up to 21% in the $CD8^-$ memory population to almost none in the $CD8^+$ naive population. The percentage of antigen-expanded cells that stained positive for IL-4 was far less, ~2%, in the $CD8^-$ memory population and was essentially nonexistent in the naive $CD8^-$ or $CD8^+$ populations.

We have previously documented by magnetic-bead CD8- and CD4-depletion experiments using these restimulation techniques that ~80%–90% of the soluble IFN-γ measured in the supernatants is produced by the $CD4^+$ population. The flow-based experiments here confirm those observations and also show that the IFN-γ–producing CD8 population is only ~20%–30% the size of the $CD8^-$ pool. In addition, the naive population remained relatively constant over the 10 days of expansion, as assessed by $CD45RO^-$ [21], and has only about one-fifth as many cells producing IFN-γ after PMA and ionomycin stimulation as does the $CD45RO^+$ memory cell population.

Vaccine-induced cytokine responses. In the protocol AVEG 016B, an increase in IFN-γ production by $CD8^-$ lymphocytes is apparent in vaccines 14 days after the first and second immunizations. A representative example of the IFN-γ production seen at these time points is shown in figure 3. As can be seen, there is an increase in both the number of $CD8^-$ cells producing IFN-γ and an increase in the intensity of the signal. This increase was not seen in the medium-stimulated control. This

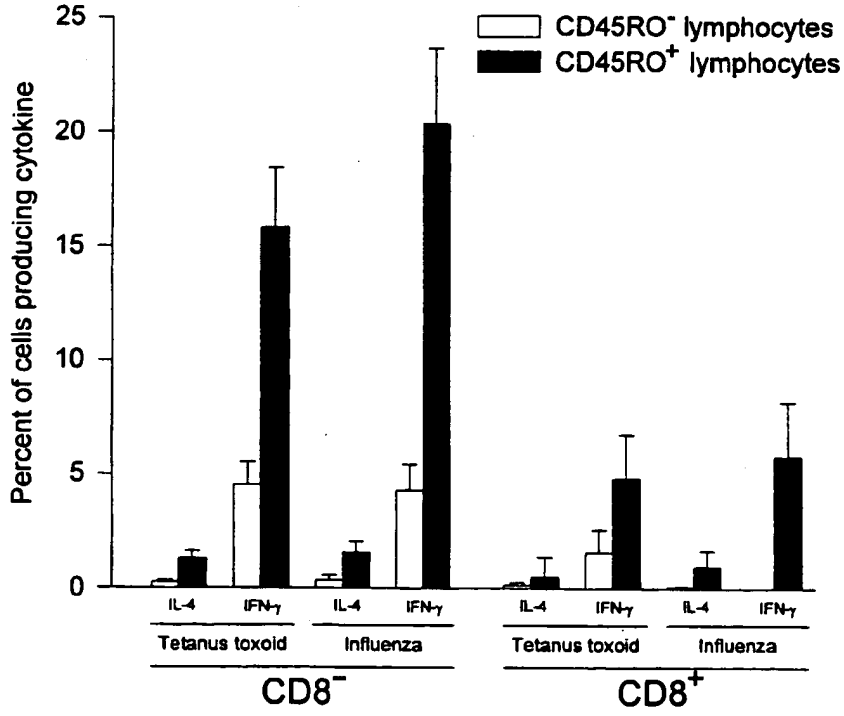

Figure 2. Relative contribution of memory ($CD45RO^+$) and naive ($CD45RO^-$) T cell subsets to cytokine staining is plotted for 2 antigen stimulations, tetanus toxoid and influenza, with control medium stimulation subtracted for each sample. Data are shown for 28 persons.

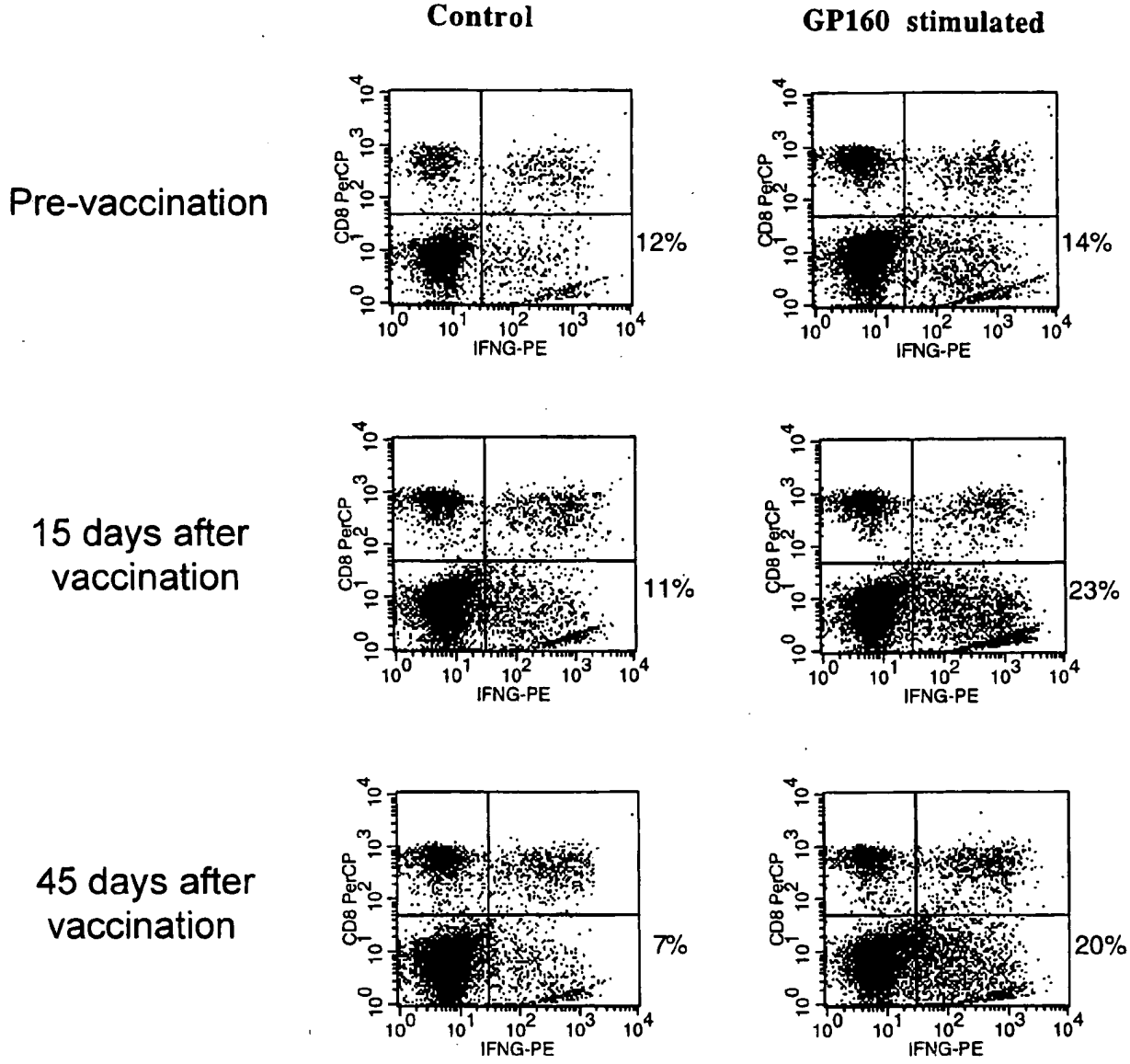

Figure 3. Representative volunteer was vaccinated with candidate gp120 immunogen, and cryopreserved peripheral blood mononuclear cells (PBMC) obtained 2 weeks after initial vaccination and 2 weeks after second vaccination (day 45) were stimulated with medium control, gp160, and tetanus antigens. After restimulation with phorbol myristate acetate and ionomycin on day 10, PBMC were surface-stained for CD45RO and CD8 and intracellularly stained for interferon (INF)-γ according to Materials and Methods. Lymphocytes are gated by light scattering characteristics, and the $CD45RO^+$ population displayed in FL2 (INF-γ) vs. FL3 channels (CD8). Percents shown refer to $CD8^-$ ($CD4^+$) cells producing INF-γ.

increase was significant after both the second and third vaccination for both the alum ($P = .04$) and QS21 ($P = .03$) groups, compared with prevaccination. In addition, there was greater IFN-γ production in the QS21 arm than in the alum arm (figure 4). There was no statistically significant increase in tetanus responses in the groups between the time points ($P = .28$).

In this trial, there was a statistically significant effect of QS21 compared with alum on lymphoproliferation (stimulation index of 39.7 vs. 11.1, respectively), neutralizing antibody (geometric mean titer of 416 vs. 28, respectively, on day 182), and delayed-type hypersensitivity testing (92% vs. 44% >5 mm to gp120 skin testing), which paralleled these IFN-γ responses. However, similar to observations in other studies, lymphocytes appear to be

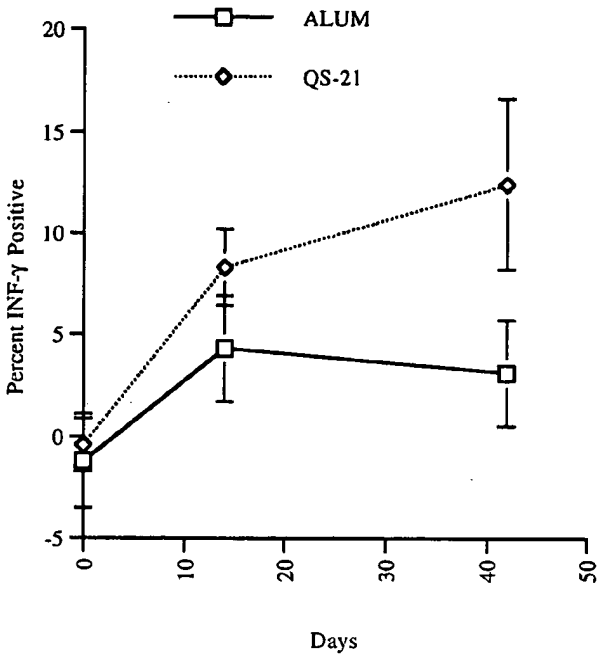

Figure 4. Interferon (INF)-γ responses are shown before vaccination and after first and third vaccinations for recipients of rsgp120 plus QS21 ($n = 6$) and rsgp 120 plus alum ($n = 7$). Data reflect mean % of $CD8^-$ T cells producing INF-γ in gp160 condition minus medium-only control.

more responsive to activation after nonspecific immunization [23]. Whether this reflects a nonspecific bystander effect is unclear. When the degree of gp120 stimulation for each individual is normalized for tetanus stimulation, there is still a significant increase in IFN-γ production by stimulated cells ($P = .04$)

Discussion

In these experiments, we have been able to show that intracellular cytokines specific for antigenic responses can be measured by intracellular staining and flow cytometric analysis. As the precursor frequency for a given antigen-specific cell is rare in the peripheral blood, we utilized a first-step antigenic expansion, followed by IL-2–supplemented medium to allow for a short-term antigen-expanded cell line to develop. Although TH1 cytokines can be measured in the supernatants of antigen-stimulated cells after 1–3 days, the number of responders as measured by flow cytometry 6 h after antigenic stimulation is quite small [24]. In our studies of antigens with lower precursor frequencies of T cells, we have been unable to determine specific responses without antigenic expansion of the population.

The cytokine responses seen with this technique of short-term cell line development may not reflect pure antigen-specific responses but may include a bystander effect of the cytokines on neighboring cells. Such a bystander amplification for IFN-γ has been shown both in vitro and in vivo [25]. However, the relevance of this model is most clearly shown in the correlation of IL-4 measured by these techniques with actual clinical outcomes. Marshall et al. [16], using a protocol similar to our own (PHA and PMA rather than PMA and ionomycin) were able to correlate IL-4 levels to outcomes in allergic patients. Bellinghausen et al. [26] used a restimulation with bound anti-CD3 and showed a close correlation between TH1/TH2 profiles and responses to bee venom therapy. In a separate study, we have also shown that the use of such culture techniques allows for the detection of TH2 responses after immunization with soluble protein HIV-1 candidate vaccines and that the timing of these responses correlates with the timing of antibody responses [21].

Are such restimulation steps really necessary for the detection of accurate TH1/TH2 profiles? Most previously published studies of intracellular staining have used either mitogens or superantigens to characterize the cytokine phenotypes of PBMC or lymph node cells. Conditions that result in a large, ongoing antigenic stimulation, such as severe viral infections, HIV-1 infection, schistosomiasis, filariasis, or allergic diatheses, might lend themselves to cytokine phenotyping by flow cytometry without the need for an expansion step. For example, Waldrop et al. [24] were able to measure CMV-specific responses in HIV-1 infected persons by stimulating PBMC with CMV antigen and measuring responses by flow cytometry 4–6 h later. By using a gating strategy that employed intracellular staining of activated cells using CD69, they were able to show that up to 0.5% of such cells produced IFN-γ. However, since surface expression of CD69 does not occur after antigen stimulation until ~48 h [27], these investigations were likely measuring ongoing effector responses in contrast to the overall T cell memory pool. In our case, in which we wish to show the potential memory immune response after vaccination, the level of ongoing stimulation is likely too small to measure directly in effector cell populations. Other techniques, such as ELISPOT or polymerase chain reaction, may be able to determine these responses with greater sensitivity but suffer in the lack of ability to accurately quantitate such responses. It is, however, possible that our techniques allow stimulated effector cells to undergo apoptosis and that this population was missed by our techniques.

IFN-γ and IL-4 were chosen to measure the TH1/TH2 profile induced by antigenic expansion. Although other cytokines could have been used to represent the TH1/TH2 profile, in most studies, these two lymphokines have been the clearest and most representative markers of the two phenotypes. In addition, the mutually exclusive production of cytokines by a single cell has been most clearly shown for these two markers [28]. In contrast, simultaneous staining for IL-2 and IL-4, IL-10 and IFN-γ, or IL-5 and IFN-γ, which have also been used to represent the TH1/TH2 paradigm, have been shown to occur with high frequency [22, 28, 29].

Our gating strategy of using CD8 and CD45 as the surface markers served two purposes. It first allowed us to separate our cytokine responses by the T cell subset responsible for producing cytokines and allowed for potential correlation of these subsets independently with other markers after vaccination, such as $CD8^+$ cytotoxic T lymphocyte responses or $CD4^+$ proliferative responses. This also allows for the detection of TH2 predominance in a CD4 population when TH1 may predominate in the CD8 subset. This response, which may be needed to elicit high levels of antibodies simultaneous with an activated CTL population, may be obscured when measuring the CD4 and CD8 responses as total PBMC. The use of the CD45 gate was used to increase the probability of detecting changes in IL-4 production by the CD4 memory subset. As previous studies have shown that the majority of IL-4 is produced by memory cells, gating on $CD45RO^+$ cells should increase the percentage of IL-4–producing cells [30]. However, more recent reports have documented large production of IL-4 by a $CD45RA^+$ cell subset that are memory cells, as determined by the loss of the CD62L (L-selectin) surface marker. This may eventually prove to be a more useful gate for determining the IL-4 production in some study populations.

These techniques were applied to a trial in which the novel adjuvant QS21 was used along with a low dose of a well-studied candidate HIV-1 vaccine (recombinant soluble MN gp120). The dose of gp120 was specifically chosen to be suboptimal for antibody production in the alum arm. Consistent with results in mouse and primate studies, the QS21 led to at least a 1.5 log dose sparing effect as measured by either neutralizing or binding antibody, improved lymphoproliferation, and greater responses on delayed type hypersensitivity skin testing. When we analyzed the $CD4^+$ responses, we found that the group receiving QS21 had greater IFN-γ production at early time points. This contrasted with the inability to measure significant INF-γ using supernatants collected 5 days after stimulation. Thus, these techniques may have advantages over other cytokine-measurement methods. They allow the specific cell populations to be phenotyped, they do not require precise counting of the readout cells (as this is performed by the cytometer), and they allow the measurement of CD4- or CD8-specific responses without having to physically remove one of these populations during the in vitro culture stage. Further applications of these techniques to the measurement of CD4 and CD8 cytokine responses in the context of HIV-1 vaccine trials is ongoing.

Acknowledgments

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Marking IL-4-producing cells by knock-in of the IL-4 gene

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Keywords: cytokines, T_h1/T_h2, transgenic/knockout

Abstract

IL-4 is a cytokine which can be expressed by a number of cell types including T_h2 cells, mast cells and a population of $CD4^+$ $NK1.1^+$ NK T cells. Although phenotypic markers exist for identifying each of these cell types, there is at present no known cell surface marker common to all IL-4-producing cells. Using gene targeting in embryonic stem cells, we have modified the IL-4 locus by knock-in of a transmembrane domain to generate mice that express a membrane-bound form of IL-4 (mIL-4). Flow cytometry using an IL-4-specific mAb allowed the detection of IL-4-secreting T_h2 cells, mast cells and NK T cells from mIL-4 mice. Furthermore, the analysis of immune responses in mIL-4 mice following immunization with anti-CD3 and anti-IgD has allowed us to identify distinct subpopulations of IL-4-producing NK T cells. Thus, the expression of IL-4 in a membrane-bound form provides a novel method for the identification and characterization of IL-4-producing cells.

Introduction

IL-4 is a 20 kDa glycoprotein originally identified by its ability to support the growth and differentiation of B lymphocytes co-stimulated with submitogenic doses of anti-Ig (1). IL-4 is now known to elicit a wide variety of responses by multiple cell types. For example, IL-4 has been shown to promote the differentiation of B lymphocytes and their switch to the production of IgE and IgG1 isotypes (2,3), and to be important for the development of cytotoxic T lymphocytes (CTL) (4,5). In addition, IL-4 is critical for the differentiation of T_h2 cells (6,7).

IL-4 was initially described as a T cell-derived cytokine (8) and its expression is one of the criteria used to distinguish T_h2 cells from IFN-γ-secreting T_h1 cells (9). Like many cytokines, however, IL-4 is now recognized to be produced by multiple cell types. For example, IL-4 has been shown to be secreted by mast cells (10), CTL (11,12) and γδ T cells (13). In addition, a population of $CD4^+$ $NK1.1^+$ NK T cells has been shown to promptly produce large amounts of IL-4 following TCR ligation *in vivo* (14).

Each of these IL-4-producing cell types can be identified by the expression of specific cell surface markers. For example, NK T cells are often identified by their expression of CD4 and NK1.1, while γδ T cells are defined by their expression of the γδ TCR. Recently, several cell surface markers have been identified which show selective expression on T_h2, but not T_h1 cells, including the eotaxin receptor CCR3 (15) and a molecule designated ST2L (16). Unfortunately, there is at present no known cell surface marker common to all IL-4-producing cell types. Moreover, it is not clear if the expression of molecules such as CCR3 and ST2L exactly correlates, on a cell to cell basis, with IL-4 expression in cells *in vivo*. While IL-4-producing cells can be identified by intracellular cytokine staining and flow cytometry, these procedures do not allow for the recovery of viable cells. Given these constraints in the ability to detect and manipulate those cells which are actually producing IL-4, we have used gene targeting in embryonic stem cells to modify the IL-4 locus and generate mice that express a membrane-bound form of IL-4 (mIL-4). Using an IL-4-specific mAb, we were able to detect IL-4-secreting T_h2 cells, mast cells and NK T cells from mIL-4 mice by flow cytometry. Furthermore, the analysis of immune responses in mIL-4 mice has allowed us to identify distinct subpopulations of IL-4-producing NK T cells.

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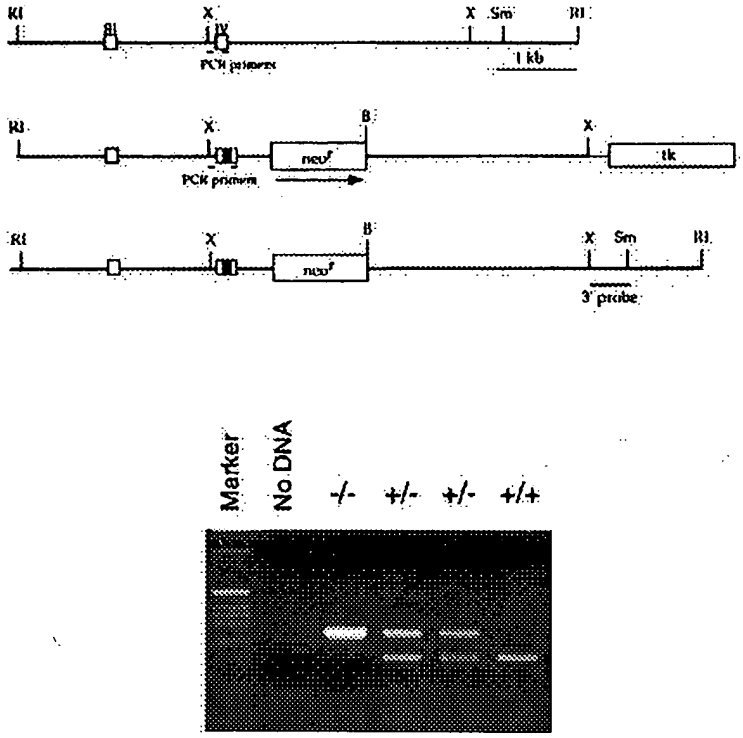

Fig. 1. Generation of mIL-4 Mice. (*Top*) The endogenous IL-4 locus (top), the targeting construct (middle) and the predicted structure of the mIL-4 allele (bottom). (*Bottom*) PCR analysis of offspring resulting from heterozygous intercross of mIL-4 mice.

Methods

Generation of membrane-bound IL-4 mice

The targeting construct used to generate a knock-in of the IL-4 locus contained exons 3 and 4 of the IL-4 gene, and was bounded by the *Eco*RI site located 1 kb upstream of exon 3 and the *Xba*I site located 3 kb downstream of exon 4 (17). Exon 4 of the murine IL-4 gene was modified by recombinant PCR to contain a transmembrane anchor. Briefly, the fourth exon of the MHC class II A_β^b gene (18), encoding the connecting peptide, the transmembrane region and the first six amino acids of the cytoplasmic tail, was inserted between the last codon and the translational stop site of the IL-4 gene. A cassette containing the neomycin resistance gene was placed 150 nucleotides downstream of the polyadenylation signal of the IL-4 gene. The targeting construct was transfected into day 3 embryonic stem cells as described (19). Homologous recombinants were identified by Southern analysis of *Eco*RI-digested DNA using the 3′ probe shown in Fig. 1; this probe detects a 7 kb fragment of the wild-type allele and a 4.5 kb fragment of the mIL-4 allele. One homologous recombinant was used to generate chimeras that passed the mIL-4 allele through the germline. Mice were genotyped using the primers shown in Fig. 1 and having the sequence: IL-4 upstream, 5′-GAG ACC CAA ATC TGT CTC AC-3′; IL-4 downstream, 5′-GTT AAA GCA TGG TGG CTC AG-3′. Unless otherwise noted, all experiments were done on 3- to 4-month-old homozygous mIL-4 mice which had been backcrossed six generations onto the BALB/c genetic background.

In vitro differentiation of T_h2 cells

Spleen cells from wild-type and mIL-4 mice were stimulated *in vitro* with 1 μg/ml plate-bound anti-CD3 (2C11) and 20 μg/ml anti-IL-12 (5C3). Twenty-four hours later, 50 U/ml IL-2 and 500 U/ml IL-4 were added. Seven days post-stimulation, cells were harvested, washed and re-stimulated with plate-bound anti-CD3 for 24 h. Cells were then stained with FITC-conjugated anti-IL-4 (11B11) (PharMingen, San Diego, CA) and analyzed by flow cytometry.

Preparation and stimulation of peritoneal mast cells

Mouse peritoneal mast cells were prepared as described (20). Briefly, the peritoneal cavity was lavaged with 10 ml modified Tyrode's buffer (2.7 mM KCl, 12 mM $NaHCO_2$, 0.4 mM NaH_2PO_4, 0.14 M NaCl, 0.1% glucose, 0.1% gelatin). The recovered peritoneal cells were washed once, resuspended in modified Tyrode's buffer (1 ml/pooled cells from 10 mice), layered onto a solution of 22.5% metrizamide and centrifuged for 12 min at 1500 r.p.m. The cell pellet was harvested, washed twice and used as a source of enriched mast cells. Enriched mast cells were then stimulated with 1 μM ionomycin for 6–8 h, stained with FITC-conjugated anti-IL-4 and analyzed by flow cytometry.

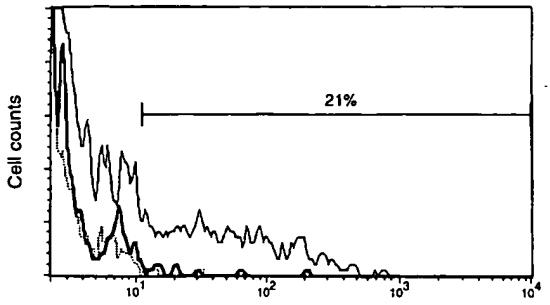

Fig. 2. Detection of IL-4-producing T_h2 cells from mIL-4 mice. Spleen cells from wild-type and mIL-4 mice were differentiated into T_h2 cells *in vitro*. Twenty-four hours after re-stimulation with plate-bound anti-CD3, wild-type (bold solid line) and mIL-4 (solid line) cells were stained with FITC-conjugated anti-IL-4 or isotype-matched control (dotted line) antibody and analyzed by flow cytometry.

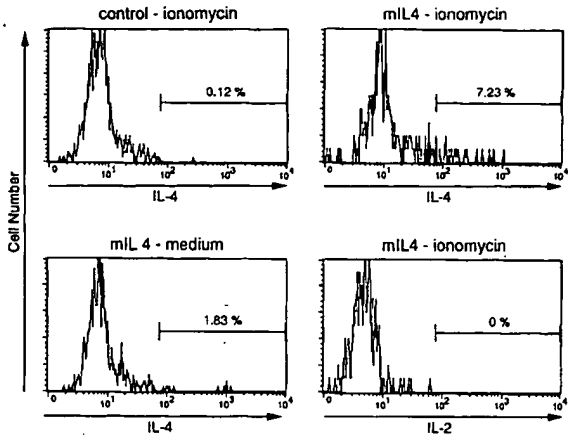

Fig. 3. Detection of IL-4-producing mast cells from mIL-4 mice. Enriched peritoneal mast cells from wild-type and mIL-4 mice were either stimulated with ionomycin or left unstimulated for 6 h. Cells were then stained with FITC-conjugated anti-IL-4, or FITC-conjugated anti-IL-2 as control, and analyzed by flow cytometry.

In vivo immunization

Anti-CD3 (100 μg per animal in 100 μl of PBS) or PBS was administered i.v. by injection into the tail vein. Spleen cells were harvested 5–6 h post-injection, stained with FITC-conjugated anti-IL-4 and analyzed by flow cytometry. Immunization with anti-IgD was performed as described (19). Spleen cells were harvested 10 days post-injection, stimulated overnight *in vitro* with plate-bound anti-CD3, stained with FITC-conjugated anti-IL-4 and analyzed by flow cytometry.

Results and discussion

Generation of mIL-4 mice

The targeting construct used to generate mIL-4 mice is shown in Fig. 1. Briefly, the fourth exon of the IL-4 gene was modified by recombinant PCR to contain the connecting peptide, the transmembrane region and the first six amino acids of the cytoplasmic tail of the MHC class II $A_\beta{}^b$ gene. A cassette containing the neomycin-resistance gene was placed 150 nucleotides downstream of the polyadenylation signal of the IL-4 gene. The targeting construct was transfected into day 3 embryonic stem cells and homologous recombinants were identified by Southern analysis as described in Methods. One clone was used to generate chimeras that passed the mIL-4 allele through the germline. Heterozygous offspring were backcrossed six generations onto the BALB/c genetic background and then intercrossed to generate homozygous mIL-4 mice (Fig. 1).

Detection of IL-4-producing T_h2 cells

To determine if mIL-4 can be detected on the cell surface of differentiated $CD4^+$ T_h2 cells, we used an *in vitro* differentiation protocol to enrich for this population. Spleen cells from wild-type and mIL-4 mice were stimulated with plate-bound anti-CD3 in the presence of IL-4 and anti-IL-12 for 7 days. Cells were then re-stimulated with plate-bound anti-CD3 for 24 h and analyzed by flow cytometry for the expression of mIL-4 using 11B11 mAb. mIL-4^+ cells could not be detected from cultures of differentiated wild-type cells (Fig. 2) nor from cultures of unstimulated spleen cells irrespective of their genotype (data not shown). In contrast, ~20% of the *in vitro* differentiated $CD4^+$ T cells from mIL-4 mice show a spectrum of positive staining with 11B11 mAb 24 h post secondary stimulation. These results demonstrate that mIL-4 expression can be used as a marker for the detection of IL-4-producing T_h2 cells. It should be noted that although nearly 75% of *in vitro* differentiated T_h2 cells from wild-type mice were positive for IL-4 when analyzed by intracellular cytokine staining (data not shown), this procedure does not allow for the recovery of viable cells. Thus, the detection of IL-4-producing cells by surface staining for mIL-4 represents a novel method for the recovery of viable IL-4-producing cells for further manipulation.

Detection of IL-4-producing peritoneal mast cells

Mast cells can secrete IL-4 following either cross-linking of FcεR or stimulation with ionomycin (21). To determine if IL-4-secreting mast cells can be detected in mIL-4 mice, peritoneal mast cells from wild-type and mIL-4 mice were generated as described in Methods. Microscopic examination revealed that >50% of the peritoneal cells prepared by this method were large granule-containing cells having a morphology consistent with being mast cells. When analyzed by flow cytometry using 11B11 mAb, ~1–2% of this mast cell enriched population from mIL-4 mice stained positive at baseline (Fig. 3). Strikingly, >7% of the cells became positive for mIL-4 expression after only 6 h of stimulation with ionomycin. Flow cytometric analysis of ionomycin-activated cells from mIL-4 mice with an IL-2-specific antibody, or of ionomycin-activated cells from wild-type mice with 11B11 mAb, failed to reveal specific staining. Thus, these data demonstrate that IL-4-producing mast cells from mIL-4 mice can be detected by flow cytometry using 11B11 mAb.

Detection of IL-4-producing NK T cells

NK T cells represent a small percentage of splenic lymphoid cells (~1%) but secrete large amounts of IL-4 upon ligation

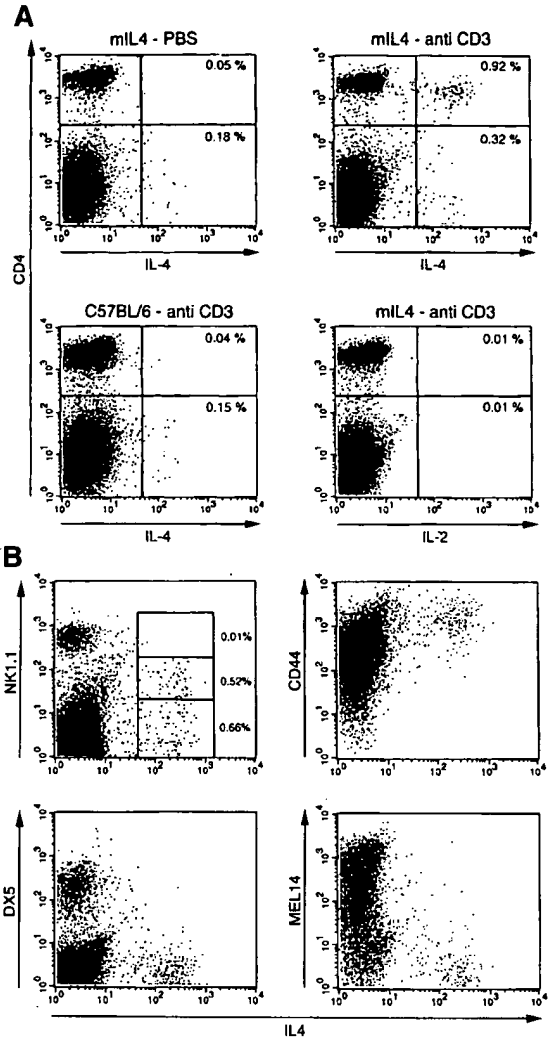

Fig. 4. Detection of IL-4-producing NK T cells from mIL-4 mice and expression of phenotypic markers. Spleen cells from wild-type C57BL/6 and (BALB/c×C57BL/6)F_1 mIL-4 mice were isolated 6 h after i.v. injection with anti-CD3 or PBS and analyzed by flow cytometry using antibodies to the indicated cell surface markers.

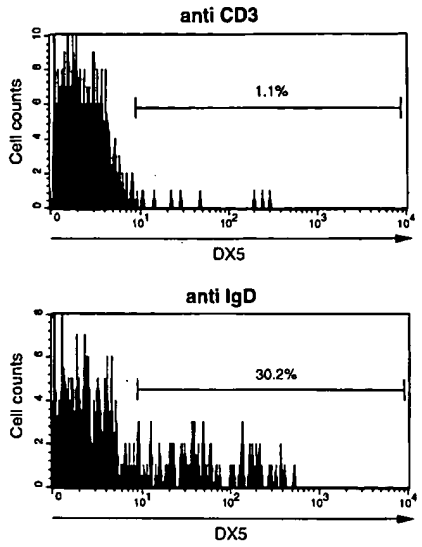

Fig. 5. DX5 expression on IL-4-producing NK T cells from anti-CD3 and anti-IgD Immunized mIL-4 mice. Spleen cells from wild-type and mIL-4 mice were either isolated 6 h after i.v. injection with anti-CD3 or harvested 10 days post-immunization with anti-IgD and stimulated overnight *in vitro* with plate-bound anti-CD3, and analyzed by flow cytometry for the expression of mIL-4 and DX5.

of their TCR by anti-CD3 *in vivo* (14). These cells are most easily identified by their cell surface expression of both CD4 and NK1.1. Since the mIL-4 mice were on a BALB/c genetic background and this strain does not express the NK1.1 marker, we mated mIL-4 mice to C57BL/6 mice to generate (BALB/c×C57BL/6)F_1 animals which expressed the NK1.1 marker and were heterozygous for the mIL-4 allele. These F_1 mIL-4 mice and wild-type C57BL/6 controls were injected with anti-CD3 i.v. When analyzed by flow cytometry 6 h post-injection with anti-CD3, ~1% of total spleen cells obtained from F_1 mIL-4 mice stained positive with 11B11 mAb (Fig. 4A). Consistent with previous reports documenting the phenotype of splenic IL-4-secreting NK T cells (22), these mIL-4^+ cells were $CD44^{high}$, $Mel14^{low}$ (Fig. 4B). Interestingly, none of the cells expressing high levels of NK1.1 stained positive for mIL-4, but rather mIL-4^+ cells were equally divided into $NK1.1^{low}$ and $NK1.1^-$ subpopulations. This observation may be related to the recent demonstration that *in vitro*, expression of the NK1.1 marker is down-regulated on NK T cells following activation (23). Alternatively, at least some of the mIL-4^+ $NK1.1^-$ cells may represent memory T_h2 cells. Nevertheless, these results demonstrate that mIL-4 expression can be used as a marker for IL-4-producing NK T cells.

In addition to their role in anti-CD3-induced IL-4 production, NK T cells are also thought to be the predominant producers of IL-4 following immunization with the polyclonal stimulus anti-IgD (24). CD1-deficient mice lack the major population of NK T cells and do not acutely produce IL-4 following *in vivo* stimulation with anti-CD3 (25–27). Nevertheless, these mice generate normal IgE antibody responses following administration of anti-IgD. These observations suggest that separate subpopulations of IL-4-secreting NK T cells, as defined by their ability to respond to different activating stimuli such as anti-CD3 or anti-IgD, may exist. To test this hypothesis, wild-type and mIL-4 mice were immunized with anti-IgD. Spleen cells were harvested 10 days after immunization and re-stimulated with plate-bound anti-CD3 *in vitro* for 24 h. Similar to that seen following injection of anti-CD3 *in vivo*, flow cytometric analysis of spleen cells from anti-IgD immunized mIL-4 mice demonstrated that ~1% of the cells were mIL-4^+ and, of these, almost all were $CD3^+$ $CD4^+$ $CD44^{high}$ $Mel14^{low}$ (data not shown). Since, mIL-4 mice on the BALB/c genetic background do not express the NK1.1 marker, we examined the expression of the pan-NK cell marker DX5 on mIL-4^+ cells. Approximately 30% of the mIL-4^+ spleen cells derived from anti-IgD injected mIL-4 mice were positive for the DX5

marker (Fig. 5). In contrast, almost none of the mIL-4$^+$ spleen cells derived from anti-CD3-injected mIL-4 mice expressed the DX5 marker (Fig. 4B and 5). Thus, these data suggest that there are at least two populations of IL-4-producing NK T cells; those that are DX5$^-$ and stimulated in response to anti-CD3, and those that are DX5$^+$ and activated in response to anti-IgD. While it is possible that the IL-4-producing DX5$^-$ NK T cells seen in anti-IgD-injected mice are the same population of IL-4-producing NK1.1$^{low/-}$ cells seen in anti-CD3-injected mice, the (BALB/c×C57BL/6) F_1 mIL-4 mice did not respond well to anti-IgD injection, thus preventing a direct test of this hypothesis. Nevertheless, these results demonstrate that IL-4-producing NK T cells are a heterogeneous population, and may explain the differences seen in the ability of CD1-deficient mice to mount acute versus chronic IL-4 responses to immunization with anti-CD3 and anti-IgD respectively.

In this report, we have shown that multiple IL-4-secreting cell types from mIL-4 mice, including T_h2 cells, mast cells and NK T cells, could be detected by flow cytometry using an IL-4-specific mAb. Since the expression of the mIL-4 allele is driven by the endogenous IL-4 promoter in the context of its normal location in the genome, the transcription of this gene is regulated in a manner that is both quantitatively and qualitatively identical to the wild-type allele. Thus, knock-in of the IL-4 locus represents the most physiological approach to mark, track and/or sort IL-4-producing cells given the lack of a natural marker. mIL-4 mice will allow the easy identification and purification of IL-4-producing cells for the study of their roles in animal models of disease.

Acknowledgements

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Abbreviations

CTL	cytotoxic T lymphocyte
mIL-4	membrane-bound IL-4

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A new in vitro model for studying human T cell differentiation: (H1)/T(H2) induction following activation by superantigens.

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A new T(H1)/T(H2) in vitro model for mechanistic studies and drug screenir human T cells was established working with ficoll-separated PBMCs or elutr lymphocytes cultured in serum-free medium. Human T cells could be kept vi and reactive in this medium for several months. In this model, superantigens were used to activate exclusively those T cell clones which were known to e specifically SA-binding Vbeta-chains of the T cell receptor. It was possible t identify the activated SA-specific T cells among the whole T cell population using monoclonal antibodies against these Vbeta-chains and to follow respor involving receptor regulation and cytokine expression. The flow cytometric analysis revealed, that SA exposure caused an upregulation of the IL-2 recep selectively in the SA-specific subpopulation. Only the T cells of this subpopulation could be shifted towards T(H1) or T(H2) differentiation, whic determined by the distribution of IFN-gamma and IL-4 positive cells. Regula of IFN-gamma could be detected by flow cytometry after 18 h and that of IL the third day of cell culture. The differentiation status could be influenced by various measures: T(H1) shifts were achieved in the presence of IL-12 and a IL-4, whereas, T(H2) shifts were induced more slowly with monocyte-reduc elutriated lymphocytes in the presence of IL-4, IL-6, anti-IL-12, 1alpha,25-dihydroxy-vitamin-D3 or combinations thereof. It was found that sialidase stimulated whereas TGF-beta and pentoxifylline suppressed both kinds of T response. The T(H1)/T(H2) differentiation persisted for several weeks after primary activation if cells were expanded in IL-2 containing serum-free cultu medium. Therefore, this human T(H1)/T(H2) in vitro model should be ideal studying early and late events of infection, allergy, and autoimmunity as well for investigating the cellular interactions involved. In addition, the early dete of the response pattern makes this model potentially useful for drug screenin

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Peptides and Proteins); 0 (Interleukin-1); 0 (Lipopolysaccharides); 0 (RNA, Messenger); 0 (Tumor Necrosis Factor-alpha); 1404-26-8 (Polymyxin B); 82115-62-6 (Interferon Type II)
Record Date Created: 19971125
Record Date Completed: 19971125
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17/6/11 (Item 11 from file: 159)
02386035 98019116 PMID: 9358084
Helicobacter pylori induces proinflammatory cytokines and major histocompatibility complex class II antigen in mouse gastric epithelial cells.
Oct 1997

17/6/12 (Item 12 from file: 155)
12028171 PMID: 9317031
Differential stimulation of interleukin-12 (IL-12) and IL-10 by live and killed Helicobacter pylori **in vitro and association of IL-12 production with gamma** interferon **-producing T cells in the human gastric mucosa.**
Oct 1997

17/6/13 (Item 13 from file: 144)
13791372 PASCAL No.: 98-0505795
Effects of cytokines, without and with Helicobacter pylori components, on mucus secretion by cultured gastric epithelial cells
1998

17/6/14 (Item 14 from file: 159)
02380720 97461350 PMID: 9317031
Differential stimulation of interleukin-12 (IL-12) and IL-10 by live and killed Helicobacter pylori **in vitro and association of IL-12 production with gamma** interferon **-producing T cells in the human gastric mucosa.**
Oct 1997

17/6/15 (Item 15 from file: 34)
06248904 Genuine Article#: YE204 Number of References: 34
Title: Helicobacter pylori induces proinflammatory cytokines and major histocompatibility complex class II antigen in mouse gastric epithelial cells (ABSTRACT AVAILABLE)
Publication date: 19971000

17/6/16 (Item 16 from file: 144)
11983357 PASCAL No.: 95-0169701
Gastric juice neopterin in Helicobacter pylori infection : Pathogenesis and host response in Helicobacter pylori infection
1995

DIALOG(R)File 155:MEDLINE(R)

11340496 PMID: 8648119

Helicobacter **-specific cell-mediated immune responses display a predominant Th1 phenotype and promote a delayed-type hypersensitivity response in the stomachs of mice.**

Mohammadi M ; Czinn S; Redline R; Nedrud J
Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA.

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Jun 15 **1996** , 156 (12) p4729-38, ISSN 0022-1767 Journal Code: 2985117R
Contract/Grant No.: DK 46461; DK; NIDDK; HL 37117; HL; NHLBI
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Subfile: AIM; INDEX MEDICUS; AIDS/HIV

Studies regarding the nature of cell-mediated immunity in **Helicobacter pylori** infection and its role in pathogenesis have yielded controversial results. To address this issue in a controlled manner, we have employed the well-characterized **Helicobacter** felis-mouse model. Immunized/challenged and nonimmunized/infected mice were evaluated for cellular proliferation, gastric inflammation, and cytokine and Ab production at various times after infection. We observed two types of cell-mediated immune responses depending on the nature of the Ag preparation. The first response is a **Helicobacter** -independent response, present in all experimental groups, which is directed toward Ags such as urease and heat shock proteins. The second is a **Helicobacter** -dependent cellular response restricted to mice previously exposed to **Helicobacter** Ags either by immunization or infection. This response was not seen in noninfected controls. The **Helicobacter** -dependent cellular response had a Th1 phenotype, as either infected or immunized/challenged mice demonstrated local and systemic production of IFN-gamma and undetectable levels of IL-4 or IL-5. Cellular proliferation correlated with the severity of gastric inflammation in both immunized/challenged (protected) and nonimmunized/infected mice. Finally, in vivo neutralization of IFN-gamma resulted in a significant reduction of gastric inflammation in H. felis-infected, as well as immunized/challenged, mice. This treatment also revealed the presence of Th2 cells, restricted to immunized/challenged mice, as demonstrated by local and systemic production of IL-4 in these mice. These data demonstrate that **Helicobacter** infection and/or immunization stimulate a predominantly Th1-type, Ag-specific response and promote a local delayed-type hypersensitivity response in the stomach that may be inhibited by depletion of IFN-gamma.

Tags: Female; Research Support, U.S. Gov't, P.H.S.
Descriptors: ***Helicobacter** --immunology--IM; *Hypersensitivity, Delayed --immunology--IM; *Lymphocyte Activation; *Stomach--immunology--IM; *Th1 Cells--immunology--IM; Animals; Gastritis--immunology--IM; Immunity, Cellular; Interferon Type II--physiology--PH; Mice; Mice, Inbred C57BL; Urease--immunology--IM
CAS Registry No.: 82115-62-6 (Interferon Type II)
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Record Date Created: 19960725
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DIALOG(R)File 155:MEDLINE(R)

11328202 PMID: 8967489

Mononuclear cells and cytokines stimulate gastrin release from canine antral cells in primary culture.

Lehmann F S ; Golodner E H; Wang J; Chen M C; Avedian D; Calam J; Walsh J H; Dubinett S; Soll A H
Center for Ulcer Research and Education, Gastroenteric Biology Center,

WEST Search History

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1. 6797493. 01 Oct 01; 28 Sep 04. Fc fusion proteins of human granulocyte colony-stimulating factor with increased biological activities. Sun; Lee-Hwei K., et al. 435/69.7; 435/325 435/358 530/350. C07K016/00 C07K014/00 C12N005/10.

☐ 2. 6761888. 26 May 00; 13 Jul 04. Passive immunization treatment of Alzheimer's disease. Schenk; Dale B.. 424/130.1; 530/300 530/350 530/387.1. C07K016/00 C07K016/18 A61K039/00.

☐ 3. 6699472. 08 Mar 01; 02 Mar 04. Method of treating allergic disorders. Jardieu; Paula M., et al. 424/133.1; 424/130.1 530/387.1 530/387.3. A61K039/395.

☐ 4. 6692745. 28 Jan 00; 17 Feb 04. Compositions and methods for inhibition of HIV-1 infection. Olson; William C., et al. 424/160.1; 424/184.1 424/185.1 424/208.1 435/7.1 530/387.3. A61K039/42 A61K039/00 G01N033/53.

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☐ 8. 6187748. 07 Jun 95; 13 Feb 01. Uses of CD4-gamma2 and CD4-IgG2 chimeras. Maddon; Paul J., et al. 514/12; 435/69.7 536/23.4. A61K038/00 C12N015/62.

☐ 9. 6130042. 05 Mar 98; 10 Oct 00. Compositions and methods for diagnosing periodontal disease. Diehl; Scott R., et al. 435/6; 435/7.1 435/91.2 435/91.52. C12Q001/68 C07H021/04 C12P019/34.

☐ 10. 5700465. 05 Jan 94; 23 Dec 97. Bovine serum and bovine IgG as preventives and therapeutives for bovine mastitis. Tao; Weng, et al. 424/130.1; 424/163.1 424/164.1 424/165.1 424/176.1 530/387.1 530/389.1 530/389.5. A61K039/395 A61K039/40 C07K016/00 C07K016/18.

☐ 11. 5660828. 06 Jan 94; 26 Aug 97. Method of treating autoimmune and/or viral-induced diseases that are mediated by CD8 phenotype T cells. Rodriguez; Moses, et al. 424/154.1; 424/85.1 530/350. A61K039/395 A61K045/05 C07K001/00 C07K014/00.

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☐ 14. 4500637. 19 Jul 82; 19 Feb 85. Prevention of graft versus host disease following bone marrow transplantation. Neville, Jr.; David M., et al. 424/140.1; 424/154.1 424/182.1 424/183.1 435/29 436/548 436/824 514/2 530/362 530/377 530/388.75 530/391.7 530/403 530/405 530/806 530/809 530/868. A61K039/00 A01N001/02 C12N015/00.

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L9: Entry 21 of 32 File: USPT Jun 1, 1999

DOCUMENT-IDENTIFIER: US 5908861 A
TITLE: Methods for treating inflammation and inflammatory disease using pADPRT inhibitors

Brief Summary Text (5):
Malignant growth and inflammatory processes share the activation of certain cellular signal transduction pathways, e.g., MAP kinase; Kyriakis et al., 1996, "Sounding the alarm: protein kinase cascades activated by stress and inflammation," J. Biol Chem. 271:24313-24316; Ferrell, J E, 1996, "Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs," TIBS 21:460-466. Chronic inflammation frequently leads to carcinogenic transformation, as demonstrated, for example, in the case of the intestinal epithelium; Kawai et al., 1993, "Enhancement of rat urinary bladder tumorigenesis by lipopolysaccharide-induced inflammation," Cancer Res. 53:5172-5; Rosin et al., 1994, "Inflammation, chromosomal instability, and cancer: the schistosomiasis model," Cancer Res. 54 (7 Suppl):1929s-1933s; Choi et al., 1994, "Similarity of colorectal cancer in Crohn's disease and ulcerative colitis: implications for carcinogenesis and prevention," Gut 35:950-4. Based on the connection between chronic inflammation and carcinogenic transformation, the aim of the present study was to investigate whether INH.sub.2 BP affects the course of the inflammatory process in vitro and in vivo. In our study, the production of multiple proinflammatory mediators was induced by bacterial lipopolysaccharide (endotoxin, LPS). LPS is known to induce a multitude of cellular reactions and triggers a systemic inflammatory response. LPS-induced pro-inflammatory mediators include tumor necrosis factor alpha (TNF), interleukin-1, interferon-gamma, whereas antiinflammatory mediators include interleukin-10 (IL-10) and interleukin-13; Deltenre et al., 1995, "Gastric carcinoma: the Helicobacter pylori trail," Acta Gastroenterol Belg. 58:193-200; Beutler, 1995, "TNF, immunity and inflammatory disease: lessons of the past decade," J. Invest. Med. 42:227-35; Liles et al., 1995, "Review: nomenclature and biologic significance of cytokines involved in inflammation and the host immune response," J. Infect Dis. 172:1573-80; Giroir, 1993, "Mediators of septic shock: new approaches for interrupting the endogenous inflammatory cascade," Critical Car. Med. 21:780-9. As a consequence of the production of these inflammatory cytokines, LPS initiates the production of inflammatory free radicals (oxygen-centered, such as superoxide, and nitrogen-centered radicals, such as nitric oxide [NO]) and of prostaglandins; Nathan, 1992, "Nitric oxide as a secretory product of mammalian cells," FASEB J. 6:3051-3064; Vane, J. R., The Croonian Lecture 1993, "The endothelium: maestro of the blood circulation," Proc. Roy. Soc. Lond B 343:225-246; Szabo, C.; 1995, "Alterations in the production of nitric oxide in various forms of circulatory shock," New Horizons 3:3-32. The production of NO in inflammation is due to the expression of a distinct isoform of NO synthase (iNOS), while the production of inflammatory cytokines is explained by the expression of a distinct isoform of cyclooxygenase (cyclooxygenase-2, COX-2); Nathan, 1992, "Nitric oxide as a secretory product of mammalian cells," FASEB J. 6:3051-3064; Vane, J. R., The Croonian Lecture 1993, "The endothelium: maestro of the blood circulation," Proc. Roy. Soc. Lond B 343:225-246; Szabo, C.; 1995, "Alterations in the production of nitric oxide in various forms of circulatory shock," New Horizons 3:3-32. iNOS, COX-2, as well as the above mentioned pro-inflammatory cytokines and free radicals which play an

important role in the LPS-induced inflammatory response; ; Nathan, 1992, "Nitric oxide as a secretory product of mammalian cells," FASEB J. 6:3051-3064; Vane, J. R., R., The Croonian Lecture 1993, "The endothelium: maestro of the blood circulation," Proc. Roy. Soc. Lond B 343:225-246; Szabo, C.; 1995, "Alterations in the production of nitric oxide in various forms of circulatory shock," New Horizons 3:3-32. Moreover, NO (or its toxic byproduct, peroxynitrite), has been implicated as a key mediator leading to the transformation of the inflammatory response into a carcinogenic process; Bartsch et al., 1994, "Endogenously formed N-nitroso compounds compounds and nitrosating agents in human cancer etiology," Pharmacogenetics 2:272-7; Liu et al., 1992, "Woodchuck hepatitis virus surface antigen induces NO synthesis synthesis in hepatocytes: possible role in hepatocarcinogenesis.," Carcinogenesis 15:2875-7; Ohshima et al., 1994, "Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis," Mutation Res. Res. 305:253-64. In the current studies, we have first investigated whether treatment with INH.sub.2 BP affects the production of the inflammatory mediators tumor necrosis factor alpha [TNF], interleukin-10, interleukin-6, NO, and prostaglandin in vivo, in LPS-induced models of inflammation.

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L9: Entry 16 of 32 File: USPT Mar 30, 2004

DOCUMENT-IDENTIFIER: US 6713062 B1
**** See image for Certificate of Correction ****
TITLE: Acinetobacter outer membrane protein and gene sequence compositions and methods

Detailed Description Text (8):
In mouse models and humans, H. pylori is associated with an increase in serum gastrin and gastrin-expressing (G)-cells with a concomitant decrease in somatostatin-expressing D cells. This change appears to follow an increase in interferon-.gamma. expressing Th1 lymphocytes cells. Atrophy of the acid-producing parietal cells leads to metaplastic changes in the stomach. The development of atrophic gastritis leads to decreased colonization by H. pylori and increased colonization by non-H. pylori organisms.

Detailed Description Text (9):
The present invention shows that Acinetobacter causes the same histology as H. pylori on the gastric mucosa. Gastric epithelial cells were isolated by mechanical dissociation. All cell populations were analyzed by flow cytometry. Two months after mice were inoculated with H. pylori or Acinetobacter, the gastric T cell numbers doubled; whereas, an increase in the number of B cells was not observed until 3 months after infection. After 4 months of infection, there was a 3-fold increase in the number of G cells and a doubling in the number of parietal cells. A 3-fold decrease in the number of D-cells occurred in H. pylori and Acinetobacter infected mice. Plasma gastrin and IL-8 levels increased after both H. pylori and Acinetobacter infection. Furthermore, CD8+ cells producing IFN-.gamma. were elevated in the infected mice.

Detailed Description Text (438):
Flow cytometric analysis was used to phenotype (subtype) the T cell response. It was found that there was an increase in the number of CD4+, CD8+ and CD8+ lymphocytes expressing IFN-.gamma. cells in both H. pylori and Acinetobacter inoculated mice compared to uninoculated mice.

Detailed Description Text (454):
In this study, CD8+ cells expressing IFN-.gamma. increased with Acinetobacter infection. Such an observation is typical of a Th1 immune response activated during H. pylori infection (Bamford et al., 1998; Mohammadi et al., 1996; Strober et al., 1997). Furthermore, immunization against IFN-Y resulted in a reduction of gastric inflammation in H. pylori infected mice (Mohammadi et al., 1996). It is also important to note that increased IFN-.gamma. expression during H. pylori infection correlates with an increase in gastrin secretion both in vivo and in vitro (Jassel et al., 1999; Lehmann et al., 1996). The present study also correlates an increase in IFN-.gamma. expression with increased plasma gastrin and G-cell numbers. In addition, a study using isolated canine antral G cells showed stimulation in gastrin release by IFN-.gamma. (Lehmann et al., 1996).

Detailed Description Text (473):
Sambrook, Fritsch, Maniatis, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1989. Saunders et al., "Simple

sequence repeats in the Helicobacter pylori genome," Mol. Microbiol., 27:1091-8, 1998. Seery, "Achlorhydria and gastric carcinogenesis," Lancet., 338:1508-9, 1991. Sharma et al., "Intragastric bacterial activity and nitrosation before, during, and after treatment with omeprazole," Br. Med. J. (Clin. Res. Ed)., 289:717-9, 1984. Singh et al., "Gastrin gene expression is required for the proliferation and tumorigenicity of human colon cancer cells," Cancer Res., 56:4111-4115, 1996. Stockbruegger et al., "Pernicious anaemia, intragastric bacterial overgrowth, and possible consequences," Scand. J. Gastroenterol, 19:355-64, 1984. Strober et al., "Reciprocal IFN-gamma and TGF-beta responses regulate the occurrence of mucosal inflammation," Immunol. Today, 18:61-4, 1997. Sumii et al., "Expression of antral gastrin and somatostatin mRNA in Helicobacter pylori-infected subjects," Am, J. Gastroenterol., 89:1515-1519, 1994. Tang et al., Nature, 356:152-154, 1992. Terres and Pajares, "An increased number of follicles containing activated CD69+ helper T cells and proliferating CD71+B cells are found in H. pylori-infected gastric mucosa," Am. J. Gastroenterol, 93:579-83, 1998. Toh et al., "Mechanisms of disease," New Eng. J. Med., 337:1441-1448, 1997. Tomb, White, Kerlavage et al., "The complete genome sequence of the gastric pathogen Helicobacter pylori," Nature, 388:539-547, 1998. Torres et al., "Stomach as a source of colonization of the respiratory tract during mechanical ventilation: association with ventilator-associated pneumonia." Eur. Respir. J, 9:1729-35, 1996. Ulmer et al., "Heterologous Protection Against Influenza by Injection of DNA Encoding a Viral Protein," Science, 259:1745-1749, 1993. Walsh and Grossman, "Gastrin," N. Engl. J. Med., 292 pt 2:1377-1384, 1975. Wang et al., Proc. Natl. Acad. Sci. USA, 90:4156-4160, 1993. Wang et al., "Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with Helicobacter felis infection," Gastroenterology, 114:675-689, 1998. Wang et al., "Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer," Gastroenterology, 118:36-47, 2000. Weinberger et al., Science, 228:740-742, 1985. Wexler et al., "The isolation and characterisation of a major outer-membrane protein from Bacteroides distasonis," J. Med. Microbiol., 37:165-75, 1992 Whitton et al., J. Virol. 67:(1) 348-352, 1993. Wolf et al., "An Integrated Family of Amino Acid Sequence Analysis Programs," Comput. Appl. Biosci., 4(1):187-191, 1988. Yang et al., "The major outer membrane protein, CD, extracted from Moraxella (Branhamella) catarrhalis is a potential vaccine antigen that induces bactericidal antibodies," FEMS Immunol. Med. Microbiol., 17:187-99, 1997. Zavros et al., "Use of the Mediman Machine to quantify gastric epithelial cells for flow cytometry," Dig. Dis. Sci., In Press, 2000. Zhang et al., Helicobacter pylori infection on the risk of stomach cancer and chronic atrophic gastritis," Cancer Detect. Prev., 23:357-67, 1999.

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(54) Title: METHODS FOR PREDICTING AND/OR DIAGNOSING THE RISK OF GASTRIC CANCER

(57) Abstract

The present invention relates to methods of predicting the risk of developing cancer and in particular to a method for diagnosing, and/or predicting the risk of developing gastric cancer in a subject infected with *Helicobacter*.

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METHODS FOR PREDICTING AND/OR DIAGNOSING THE RISK OF GASTRIC CANCER

TECHNICAL FIELD

The present invention relates to methods of predicting the risk of developing cancer and in particular to a method for diagnosing, and/or predicting the risk of developing, gastric cancer in a subject infected with *Helicobacter pylori*.

BACKGROUND

Helicobacter pylori infection is now recognised as an essential pre-requisite for the development of gastric cancer. About 30% of the population become infected with this bacterium and commonly present with chronic gastritis. This may be complicated by gastric or duodenal ulceration, or may present as non-ulcer dyspepsia. A sizeable number of carriers are asymptomatic. However, in a small number of patients with *H. pylori,* their condition evolves through stages (including epithelial cell metaplasia and dysplasia) into neoplasia. The factors responsible for this evolution are complicated, but involve geographical, environmental and genetic parameters. Of particular importance is the host response. Current evidence supports the theory that a particular T cell response known as Th1 (characterised by production of γinterferon (γIFN) but not interleukin-4 (IL-4)) as promoting mucosal damage. Alternatively, a Th0 response can occur which includes balanced production of these cytokines (γIFN and IL-4) and which favours protection from mucosal damage. Patterns of mucosal cytokine response associated with neoplastic transformation and tumour progression have not been described.

Current Management Practice of *H. pylori* Infection

H. pylori is an essential component of the chain of events leading to chronic gastritis and peptic ulceration. Eradication of infection with antibiotics induces an 80-

90% cure rate of peptic ulceration. A widely accepted treatment paradigm is based on detection of infection using antibody assays, followed by combination antibiotic therapy without prior endoscopic diagnosis. Endoscopy, before eradication therapy is generally accepted when 'danger' symptoms (eg, severe pain, bleeding) occur, or a significant risk of gastric cancer is present. However, endoscopy is a procedure which is associated with its own risks and is to be avoided if possible.

At present, no non-invasive test exists which would allow for prediction or diagnosis of gastric cancer in patients which *Helicobacter* infection. Such a test would be particularly valuable for patients who present with relatively mild symptoms but who are identified as being in a "high risk" category and who would otherwise automatically be required to undergo an endoscopy - with its attendant risks. Even in patients who present with "danger symptoms" and who may still require an endoscopy, such a non-invasive test could be used as a complementary tool in diagnosis. This change in practice could have a significant impact on health economics.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

SUMMARY OF THE INVENTION

It has surprisingly been found that mucosal IgG2 anti-*H. pylori* antibody and γIFN levels are decreased and IL-4 levels are elevated in patients having *Helicobacter* infection when gastric cancer or precancer lesions (metaplasia and dysplasia) are present. These changes are also reflected in the blood of such patients. However, the changes are not seen in other disorders in which *Helicobacter pylori* is colonising the gastric mucosa.

According to a first aspect, the present invention provides a method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including:

a) determination of IgG2 anti-*H. pylori* antibody level in the subject;

b) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein a reduction in the level of IgG2 anti-*H. pylori* antibody in the subject compared to the control indicates the presence and/or increased risk of developing gastric cancer.

According to a second aspect, the present invention provides a method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including:

a) determination of γIFN level in the subject;

b) comparison of the γIFN level with a predetermined control γIFN level, wherein a reduction in the level of γIFN in the subject compared to the control indicates the presence and/or increased risk of developing gastric cancer.

According to a third aspect, the present invention provides a method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including:

a) determination of IL-4 level in the subject;

b) comparison of the IL-4 level with a predetermined control IL-4 level, wherein an elevation in the level of IL-4 in the subject compared to the control indicates the presence and/or increased risk of developing gastric cancer.

According to a fourth aspect, the present invention provides a method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including a combination of a method according to the first aspect and/or a method according to claim second aspect and/or a method according to the third aspect.

According to a fifth aspect, the present invention provides a method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including a combination of a method according to the second aspect and a method according to the third aspect.

Preferably, the *Helicobacter* infection is a *Helicobacter pylori* infection.

Preferably, the IgG2 anti-*H. pylori* antibody, γIFN and/or IL-4 levels are determined by detection in a sample of biological fluid such as for example blood, saliva, gastric fluid and the like.

Preferably, the measurement of IgG2 anti-*H. pylori* antibody, γINF and/or IL-4 either simultaneously provides, or can be performed simultaneously with, a method which provides an indication of *H. pylori* status.

Preferably, the IgG2 anti-*H. pylori* antibody and/or γIFN and/or IL-4 are detected by a near-subject assay. The assay may, however, also be a laboratory-based test. Preferably, the assay is an antibody assay although it will be understood that other known methods of measurement can also be effectively used. Most preferably, the assay is an ELISA .

According to a sixth aspect, the present invention provides a method of predicting the risk of, and/or diagnosing, gastric cancer in a subject having a *Helicobacter* infection by

a) determining the frequency of IgG2 anti-*H.pylori* antibody- and/or γIFN- and/or IL-4-producing cells in the subject's blood; and

b) comparison of the frequency of IgG2 anti-*H.pylori* antibody- and/or γIFN- and/or IL-4-producing cells in the subject's blood with a predetermined control level, wherein a reduction in the level of IgG2 anti-*H.pylori* antibody- and/or γIFN-producing cells and/or an elevation in IL-4-producing cells in the subject's blood indicates the presence and/or increased risk of developing gastric cancer.

It will be clear to the skilled addressee that the blood may be purified to provide an enriched white blood cell population and the white blood cell population may be further fractionated to obtain specific cell populations.

Preferably, the IgG2 anti-*H.pylori* antibody- and/or γIFN- and/or IL-4-producing cells are stimulated with *H. pylori* antigen prior to measurement of IgG2 anti-*H.pylori* antibody and/or γIFN and/or IL-4.

According to a seventh aspect, the present invention provides a method of predicting the risk of, and/or diagnosing, gastric cancer in a subject having a *Helicobacter* infection by

a) determining the frequency of IgG2 anti-*H.pylori* antibody and/or γIFN and/or IL-4-producing cells in the subject's gastric mucosa; and

b) comparison of the frequency of IgG2 anti-*H.pylori* antibody and/or γIFN and/or IL-4-producing cells in the subject's gastric mucosa with a predetermined control level,

wherein a reduction in the level of IgG2 anti-*H.pylori* antibody- and/or γIFN-producing cells and/or an elevation in IL-4-producing cells in the subject's gastric mucosa indicates the presence and/or increased risk of developing gastric cancer.

Preferably, the cells are derived from a biopsy sample.

Preferably, the IgG2 anti-*H.pylori* antibody- and/or γIFN- and/or IL-4-producing cells are detected by flow cytometry.

Control levels of IgG2 anti-*H. pylori* antibody, IL-4 and/or γIFN can be established in samples of biological fluids obtained from normal individuals, ie. those not having an established *H. pylori* infection, or they can be established in samples from subjects with *H. pylori* infection who have uncomplicated chronic gastritis or asymptomatic infection or the like. In certain cases, in which subjects are followed prospectively, control levels may be internal levels, i.e. the subject's own control levels.

The method of the present invention can also be used to diagnose and/or determine the risk of developing pre-cancer lesions such as metaplasia or dysplasia by way of measurement of IgG2 anti-*H. pylori* antibody, γIFN and/or IL-4.

It will be clear to the skilled addressee that ratios of IgG2 anti-*H. pylori* antibody, γIFN or IL-4 to other parameters such as, for example total IgG anti-*H. pylori* antibody may be useful as a predictor of, or in the diagnosis of, gastric precancerous or cancerous conditions, including situations in whch dysplasia and metaplasia are present. Refinement of the prediction and/or diagnosis of precancerous or cancerous conditions may require that specific ratios be utilised, such as the ratio of IL-4:γIFN, IgG2:total IgG or IgG2:IgG1. However, other ratios may also be useful.

In the context of the present invention, the abbreviations "γIFN" and "IFNγ" have been used interchangably in the specification to refer to the cytokine γ interferon.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Detection of IL-4 in supernatants of gastric mucosal cultures from subjects with gastric cancer or pre-cancer lesions (metaplasia or dysplasia). In uncomplicated *H. pylori* infection (or in benign peptic ulcers) a Th1 pattern of cytokine (eg, γINF) is found.

Figure 2. This figure illustrates a high level of correlation between secretion of IL-4 from mucosal biopsies, and *H. pylori* antigen stimulated blood T cells. IL-4 was not secreted from antigen stimulated T cells in untreated subjects with uncomplicated chronic gastritis and *H. pylori* infection.

Figure 3. Cytokine (IL-8, IL-4 and γIFN) production in the gastric mucosa of subjects infected with *H. pylori*..

Figure 4. IgG1 and IgG2 anti-*H. pylori* antibody levels in serum of *H. pylori*-infected subjects having various gastrointestinal disorders.

Figure 5. IgG1 and IgG2 anti-*H. pylori* antibody levels in serum of *H. pylori*-infected subjects having various gastrointestinal disorders.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention will now be described in more detail with reference to non-limiting examples.

It was previously known that total IgG anti-*H. pylori* antibody levels in blood and gastric mucosa can be used as an indicator of *H. pylori* status. In the following examples, therefore, it will be understood that, while IgG anti-*H. pylori* can be utilised as a general indicator of *H. pylori* status, the invention also relates to the measurement of the IgG2 subclass which can be used as a predictor of, or in the diagnosis of, gastric cancer.

Techniques for measurement of cytokines and antibodies in human samples are well-known in the art and protocols and reagents are readily available. Examples of some of the techniques used are indicated below as an illustration of how some measurements may be performed.

Unless indicated otherwise, standard techniques which can be ascertained from standard texts and laboratory manuals may be employed.

Example 1 Determination of cytokine and antibody levels in a blood sample

The standard assay involves coating microwells of a 96-well microtitre plate with monclonal anti-IL-4 (MoAb). After removal of antibody and washing with PBS/Tween 20, 100 uL of whole blood is added to each well containing an equal volume of AIM-V medium. After incubation for 24 hrs at 37°C, the plasma supernatant is removed for measurement of γIFN by ELISA (Figure 1). The amount of IL-4 captured by IL-4 MoAb in each well is measured by ELISA (Figures 1 and 2). IgG1 and IgG2 subclass anti-*H. pylori* levels or IgG2/IgG ratios in serum from clotted blood or plasma supernatant (above) are measured by ELISA (Figures 4, 5). All samples are stored at -80°C until assay.

Assay system for measurement of IL-4 alone or IL-4 and anti-*H. pylori* IgG antibodies at the same time.

Wells of a 96-well flat-bottomed microtitre plate are coated with 2 μg/mL of monoclonal anti-IL-4 capture antibody in sodium bicarbonate buffer pH 8.5. After removal of antibody solution, an equal volume of freshly collected whole blood is added to each well. After incubation for 24 hrs at 37°C, the plasma supernatant as removed and IL-4 bound is detected by reaction with biotinylated anti-IL-4 antibody and strepavidin-peroxidase conjugate. The amount of IL-4 is measured by colour development read in a plate reader with the appropriate standards.

On the same plate, IgG anti-*H. pylori* antibody is detected by adding the plasma supernatant to wells coated with 4 ug/mL of *H. pylori* antigens using an ELISA assay. The results are shown in Table 1.

Table 1 IL-4 production and anti-*H. pylori* IgG antibody in whole blood

Subject	IL-4 production (pg/mL)	*H. pylori* IgG (ELISA Index)
S1	42.77	0.696
S2	9.4	1.61
S3	13.49	1.86
S4	108.25	0.95
S5	9.4	1.83
S6	18.1	0.67
S7	9.4	4.32
S8	19.41	3.22
S9	56.64	3.48
S10	15.1	3.42
S11	9.4	0.12

Example 2 Frequency of IL-4 and γIFN producing cells in gastric mucosa

Gastric T cells are isolated from biopsy tissues obtained at endoscopy. The tissues is rinsed in 1mM dithiothreitol and 1mM EDTA to remove epithelial cells and intraepithelial cells before extraction of lamina propria T cells in serum-free AIM-V medium containing 40 U collagenase (Worthington Biochemical) for 2-3 hrs. The viability of the mononuclear cells after removal of undigested materials was >90% by trypan blue exclusion. Isolated gastric mononuclear cells from individual biopsies are usually too low (about 0.503×10^5 cells per biopsy) for antigen-mediated re-stimulation in bulk cultures. Therefore, IL-4 and γIFN producer frequencies in each cell isolate are determined by intracellular staining and then analysed on the FACS Vantage using 3-colour flow cytometry. Isolated gastric cells were activated with PMA and ionomycin and PMA, stained with PerCP-CD3 monoclonal antibody (Becton Dickinson) and then processed for intracellular staining with FITC-γIFN and PE-IL-4 monoclonal antibody as described above.

Unless indicated otherwise above, standard techniques which can be ascertained from standard laboratory texts were used.

Table 2 provides an example of the predictions/diagnoses which can be made on the basis of the above tests.

Table 2

IgG anti-*H. pylori* antibody +ve IL-4 -ve	low cancer risk endoscopy not indicated on age indications alone
IgG anti-*H. pylori* antibody +ve IL-4 +ve	high cancer risk needs endoscopy as early intervention
IgG anti-*H. pylori* antibody -ve IL-4 -ve	no evidence of *H. pylori* infection

Example 3 Frequency of IL-4 and γIFN producing cells in peripheral blood

Intracellular cytokine staining and detection by flow cytometry is used to estimate cytokine-producer frequencies of IL-4 and γIFN amongst different subjects. This allows comparison of results obtained from gastric biopsy tissue where analysis by limiting dilution culture following antigen re-stimulation is not possible due to low numbers of cells isolated per biopsy. Peripheral blood mononuclear cells or whole blood is activated with phorbol myristate acetate (PMA, 50 ng/mL) and 1 μM ionomycin for 4-5 hrs in the presence of 2 μM monensin, fixed, permeabilised and stained with FITC/PE labelled γIFN/IL-4 (Bectin-Dickinson). γIFN and IL-4 frequencies are then analysed by flow cytometry with matched isotype IgG control and gated for lymphocytes.

The frequencies of IL-4 and γIFN producing cells in peripheral blood from subjects with or without *H. pylori* infection are shown in Tables 3 and 4. The ratios of γIFN:IL-4 producing cells were higher in subjects infected with *H. pylori* than in non-infected subjects.

Limiting dilution analysis was used to determine quantitative estimates of the frequency of circulating IL-4 and γIFN-secreting cells in blood using short-term cultures stimulated with Hp recombinant antigen (citrate synthase of Hp 0310). A non-protective recombinant antigen Hp-0162 was used as a negative control. Cells are seeded in V-bottomed 96-well microplate using twofold dilution from 10^5 to 2.5×10^3 cells at 24 replicates per cell concentration. Cultures were stimulated with a predetermined concentration of citrate synthase or Hp 0310 antigen in the presence of rIL-2 (5 U/mL) for 3 days. Controls contained no responder cells or responder cells in medium and rIL-2 without antigen. As IL-4 is unstable an antibody capture method is used with bound

IL-4 measured by ELISA using a matched antibody pair (Endogen/CSL). γIFN production is measured in the supernatant by standard methods. Frequencies of peripheral blood mononuclear cells producing IL-4 and γIFN are calculated by maximum likelihood method using appropriately validated computer software.

Table 3 Cytokine producing cells in *H. pylori* antibody POSITIVE subjects

Subjects	γIFN(%)	IL-4 (%)	γIFN:IL-4 ratio
S1	18.3	2.5	7.3
S2	25.4	3.3	7.6
S3	26.0	11.5	2.3
S4	9.6	2.6	3.7
S5	14.8	5.6	2.6
Mean ± SE			4.7 ± 1.15*

Table 4 Cytokine producing cells in *H. pylori* antibody NEGATIVE subjects

Subjects	γIFN (%)	IL-4 (%)	γIFN:IL-4 ratio
S6	24.8	28.3	0.9
S7	8.9	3.0	3.0
S8	8.1	2.6	3.1
S9	29.9	29.1	1.0
S10	25.9	17.2	1.5
Mean ± SE			1.9 ± 0.48*

* p=0.054

Figures 1 to 5 provide results obtained utilising the tests exemplified below in studies of subjects having various gastrointestinal conditions i.e. reflux, gastritis, duodenal ulcer, gastric ulcer and gastric cancer. The Figures are self-explanatory and show that levels of

IgG2, γIFN and IL-4 can be used as predictors of, and in the diagnosis of, gastric cancer in patients having *H. pylori* infection.

Although the invention has been described with reference to specific examples, it will be appreciated by those skilled in the art that the invention may be embodied in many other forms without departing from the spirit or intent of the inventive concept.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including:

a) determination of IgG2 anti-*H. pylori* antibody level in the subject;

b) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein a reduction in the level of IgG2 anti-*H. pylori* antibody in the subject compared to the control indicates the presence and/or increased risk of developing gastric cancer.

2. A method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including:

a) determination of γIFN level in the subject;

b) comparison of the γIFN level with a predetermined control γIFN level, wherein a reduction in the level of γIFN in the subject compared to the control indicates the presence and/or increased risk of developing gastric cancer.

3. A method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including:

a) determination of IL-4 level in the subject;

b) comparison of the IL-4 level with a predetermined control IL-4 level, wherein an elevation in the level of IL-4 in the subject compared to the control indicates the presence and/or increased risk of developing gastric cancer.

4. A method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including a combination of a method

according to claim 1 and/or a method according to claim 2 and/or a method according to claim 3.

5. A method of diagnosing and/or determining the risk of developing gastric cancer in a subject with a *Helicobacter* infection, including a combination of a method according to claim 2 and a method according to claim 3.

6. A method according to any one of claims 1 to 6 wherein the *Helicobacter* infection is a *Helicobacter pylori* infection.

7. A method according to any one of claims 1 to 7 wherein the IgG2 anti-*H. pylori* antibody, γIFN and/or IL-4 levels are determined by detection of the levels in a sample of biological fluid.

8. A method according to claim 7 wherein the biological fluid is blood.

9. A method according to claim 7 wherein the biological fluid is saliva.

10. A method according to claim 7 wherein the biological fluid is gastric fluid.

11. A method according to any one of claims 1 to 10 wherein the measurement of IgG2 anti-*H. pylori* antibody, γINF and/or IL-4 either simultaneously provides, or can be performed simultaneously with, a method which provides an indication of *H. pylori* status.

12. A method according to any one of claims 1 to 11 wherein the IgG2 anti-*H. pylori* antibody, γIFN and/or IL-4 are detected by a near-subject assay.

13. A method according to any one of claims 1 to 11 wherein the assay is a laboratory-based test.

14. A method according to claim 12 or claim 13 wherein the assay is an antibody assay.

15. A method according to claim 14 wherein the antibody assay is an ELISA.

16. A method of predicting the risk of, and/or diagnosing, gastric cancer in a subject having a *Helicobacter* infection by

a) determining the frequency of IgG2 anti-*H.pylori* antibody- and/or γIFN- and/or IL-4-producing cells in the subject's blood; and

b) comparison of the frequency of IgG2 anti-*H.pylori* antibody- and/or γIFN- and/or IL-4-producing cells in the subject's blood with a predetermined control level, wherein a reduction in the level of IgG2 anti-*H.pylori* antibody- and/or γIFN-producing cells and/or an elevation in IL-4-producing cells in the subject's blood indicates the presence and/or increased risk of developing gastric cancer.

17. A method according to claim 16 wherein the blood is purified to provide an enriched white blood cell population.

18. A method according to claim 17 wherein the white blood cell population is further fractionated to obtain specific cell populations.

19. A method according to any one of claims 16 to 18 wherein the IgG2 anti-*H.pylori* antibody- and/or γIFN- and/or IL-4-producing cells are stimulated with *H. pylori* antigen prior to measurement of IgG2 anti-*H.pylori* antibody and/or γIFN and/or IL-4.

20. A method of predicting the risk of, and/or diagnosing, gastric cancer in a subject having a *Helicobacter* infection by

a) determining the frequency of IgG2 anti-*H.pylori* antibody and/or γIFN and/or IL-4-producing cells in the subject's gastric mucosa; and

b) comparison of the frequency of IgG2 anti-*H.pylori* antibody and/or γIFN and/or IL-4-producing cells in the subject's gastric mucosa with a predetermined control level,

wherein a reduction in the level of IgG2 anti-*H.pylori* antibody- and/or γIFN-producing cells and/or an elevation in IL-4-producing cells in the subject's gastric mucosa indicates the presence and/or increased risk of developing gastric cancer.

21. A method according to claim 20 wherein the cells are derived from a biopsy sample.

22. A method according to claim 20 or claim 21 wherein of IgG2 anti-*H.pylori* antibody and/or γIFN and/or IL-4-producing cells are detected by flow cytometry.

FIGURE 1

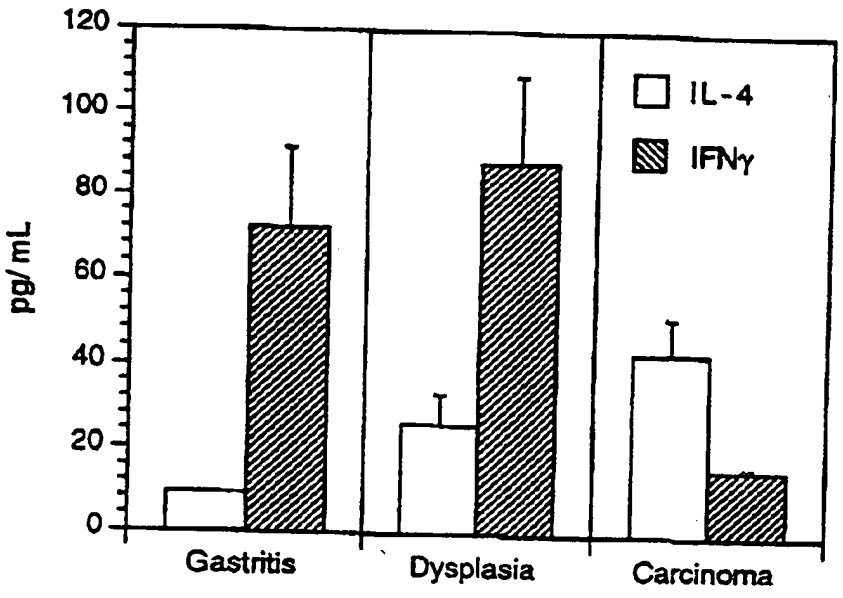
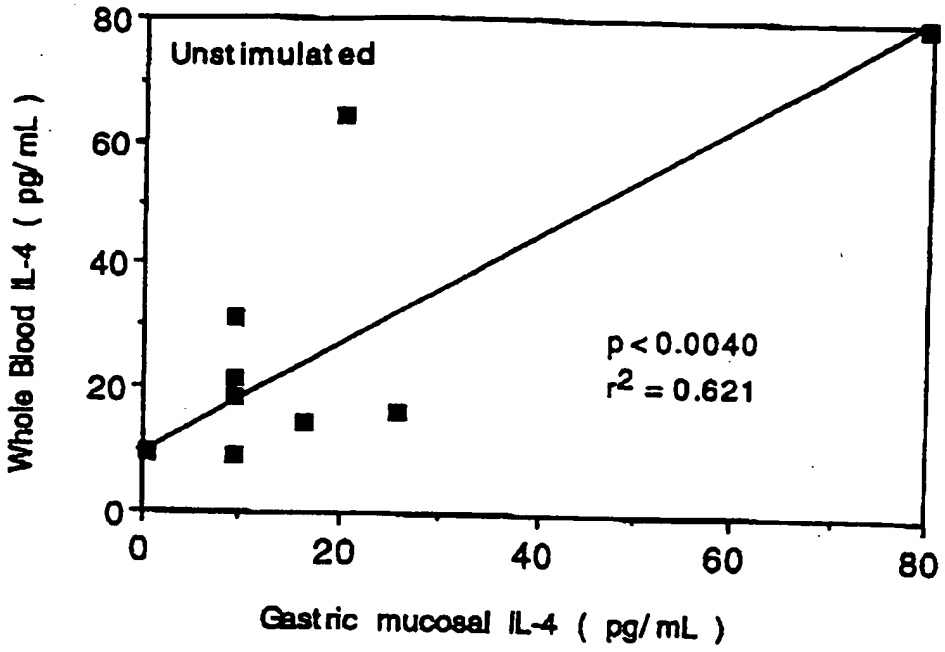

120
100
80
60
40
20
0
pg/mL
IL-4
IFNγ
Gastritis
Dysplasia
Carcinoma

FIGURE 2


Unstimulated
p < 0.0040
r2 = 0.621
Whole Blood IL-4 (pg/mL)
Gastric mucosal IL-4 (pg/mL)
0
20
40
60
80

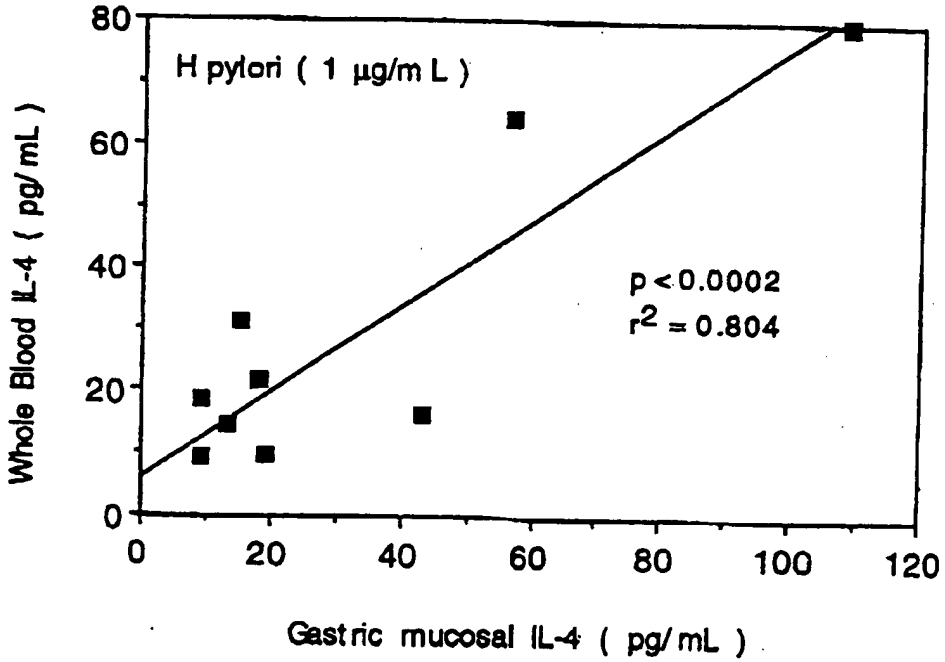

H pylori (1 µg/mL)
p < 0.0002
r2 = 0.804
Whole Blood IL-4 (pg/mL)
Gastric mucosal IL-4 (pg/mL)
0
20
40
60
80
100
120

FIGURE 3

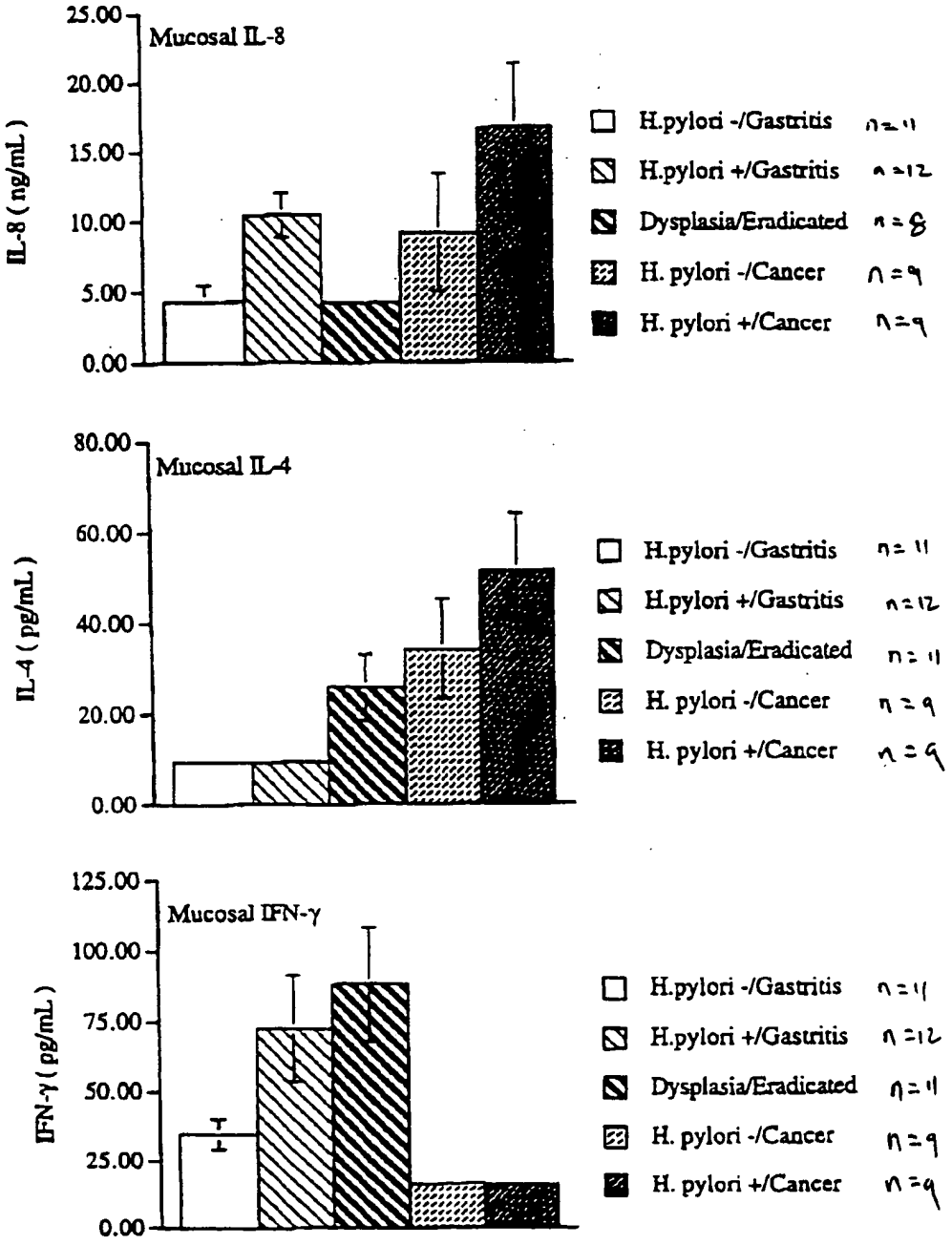

Mucosal IL-8
IL-8 (ng/mL)
25.00
20.00
15.00
10.00
5.00
0.00
H.pylori -/Gastritis n=11
H.pylori +/Gastritis n=12
Dysplasia/Eradicated n=8
H. pylori -/Cancer n=9
H. pylori +/Cancer n=9
Mucosal IL-4
IL-4 (pg/mL)
80.00
60.00
40.00
20.00
0.00
H.pylori -/Gastritis n=11
H.pylori +/Gastritis n=12
Dysplasia/Eradicated n=11
H. pylori -/Cancer n=9
H. pylori +/Cancer n=9
Mucosal IFN-γ
IFN-γ (pg/mL)
125.00
100.00
75.00
50.00
25.00
0.00
H.pylori -/Gastritis n=11
H.pylori +/Gastritis n=12
Dysplasia/Eradicated n=11
H. pylori -/Cancer n=9
H. pylori +/Cancer n=9

FIGURE 4

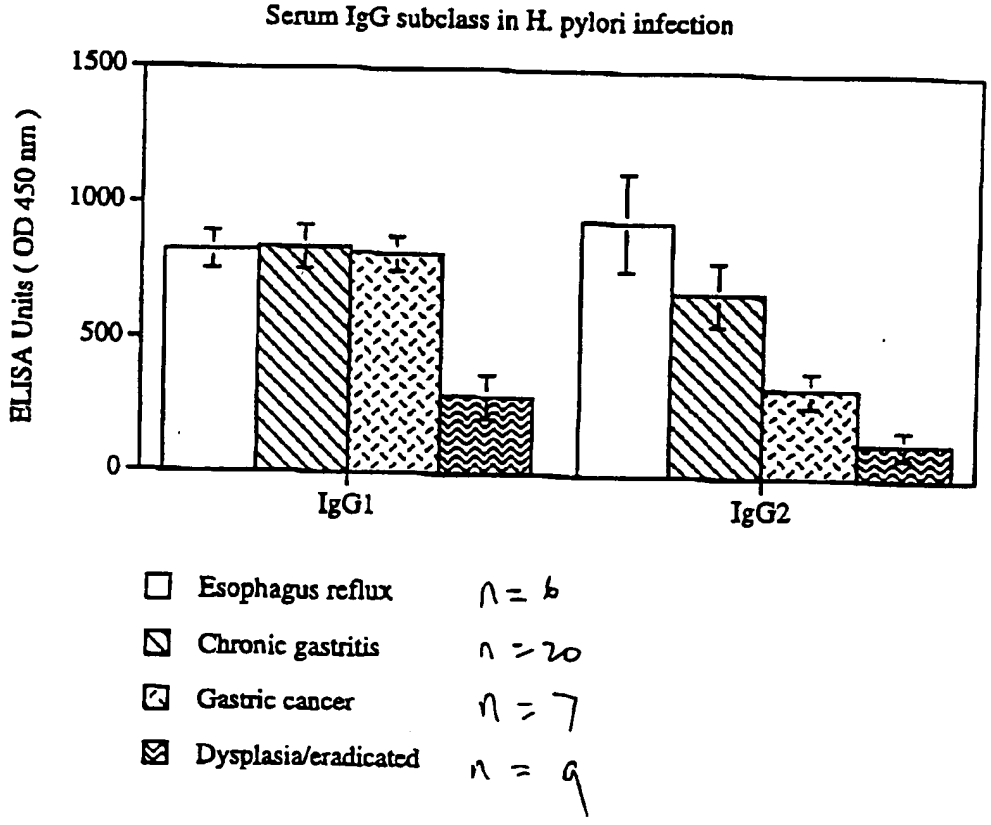
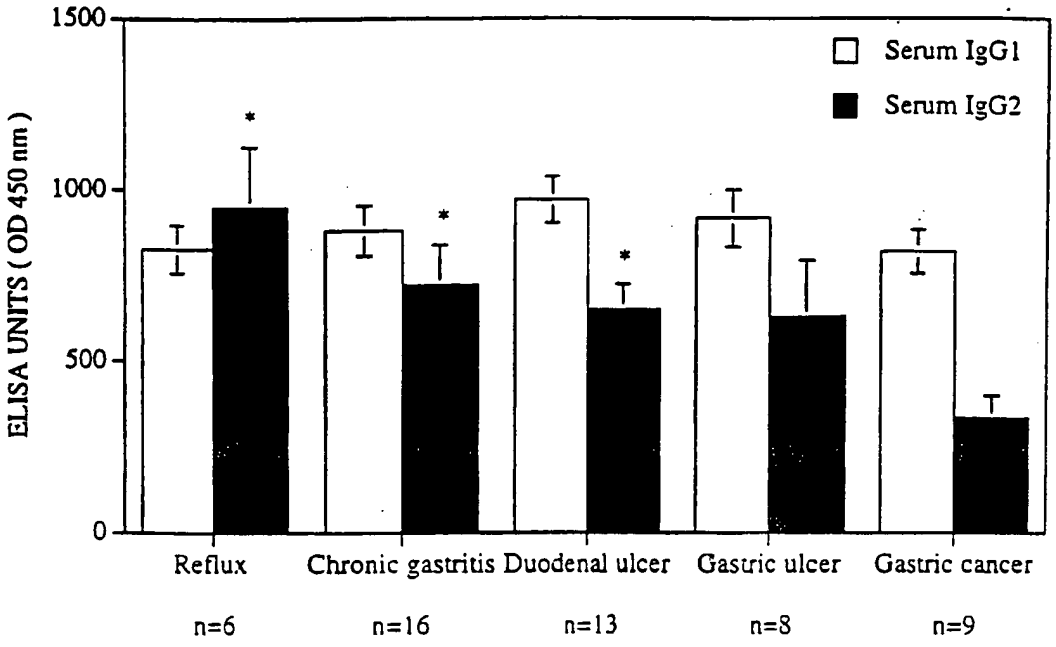

Serum IgG subclass in H. pylori infection
ELISA Units (OD 450 nm)
1500
1000
500
0
IgG1
IgG2
Esophagus reflux n = 6
Chronic gastritis n = 20
Gastric cancer n = 7
Dysplasia/eradicated n = 9

FIGURE 5


1500
1000
500
0
ELISA UNITS (OD 450 nm)
Serum IgG1
Serum IgG2
*
*
*
Reflux
Chronic gastritis
Duodenal ulcer
Gastric ulcer
Gastric cancer
n=6
n=16
n=13
n=8
n=9

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L9: Entry 27 of 32 File: DWPI Feb 26, 2004

DERWENT-ACC-NO: 2002-154775
DERWENT-WEEK: 200416

TITLE: Monitoring progress of treatment and/or eradication of Helicobacter pylori infection in a subject undergoing treatment by determining immunoglobulin G2 anti-H.pylori antibody level in saliva sample

Basic Abstract Text (1):
NOVELTY - Monitoring eradication of Helicobacter (HB) infection, comprising determining immunoglobulin G2 (IgG2) anti-H. pylori antibody level in a saliva sample, and predicting likelihood of successful eradication of HB infection in a subject to be treated or being treated for the infection involves determining levels of interleukin-4 (IL-4), interferon- gamma (IFN- gamma) and IgG in the subject, is new.

Basic Abstract Text (2):
DETAILED DESCRIPTION - Monitoring efficacy of treatment, eradication of HB infection, relapse or reinfection with HB infection and detecting unresponsiveness of a subject to treatment of HB infection, comprises determining immunoglobulin (Ig)G2 anti-H. pylori antibody level in a saliva sample, and comparing the IgG2 anti-H. pylori antibody level with a predetermined control IgG2 anti-H. pylori antibody level, where a reduction in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control, indicates eradication of HB, efficacious treatment of HB, an increase in the antibody level indicates relapse or reinfection with HB, and lack of change in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates lack of response to treatment. Predicting the likelihood of successful eradication of HB infection in a subject to be treated or being treated for the infection, comprises determining IL-4, IFN-gamma , IgG levels and/or their combinations in a sample from the subject, and comparing the levels with a predetermined control or standard level. A level of IL-4 in the sample from the subject above the control or standard IL-4 level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard IL-4 level is predictive of the likelihood of eradication failure, and a level of IFN- gamma or IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IFN- gamma or IgG above the control or standard level is predictive of the likelihood of eradication failure.

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L9: Entry 30 of 32 File: DWPI Sep 1, 2003

DERWENT-ACC-NO: 2001-025046
DERWENT-WEEK: 200465

TITLE: Diagnosis of gastric cancer, or assessment of the risk of developing it, using a largely non-invasive method which uses the level of e.g., gamma-interferon or interleukin-4, as a marker

INVENTOR: CLANCY, R L; PANG, G

PATENT-ASSIGNEE: VRI BIOMEDICAL LTD (VRIBN), ONCO ALERT PTY LTD (ONCON), ONCO ALERT HOLDING CO LTD (ONCON), CLANCY R L (CLANI), PANG G (PANGI)

PRIORITY-DATA: 1999AU-0000377 (May 14, 1999)

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PATENT-FAMILY:

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MX 2001011721 A1	September 1, 2003		000	C12Q001/68
WO 200070348 A1	November 23, 2000	E	027	G01N033/574
AU 200043862 A	December 5, 2000		000	G01N033/574
BR 200010558 A	February 19, 2002		000	G01N033/574
EP 1183540 A1	March 6, 2002	E	000	G01N033/574
CN 1355885 A	June 26, 2002		000	G01N033/574
KR 2002033093 A	May 4, 2002		000	G01N033/574
JP 2002544520 W	December 24, 2002		024	G01N033/574
US 20040157277 A1	August 12, 2004		000	G01N033/574

DESIGNATED-STATES: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
MX2001011721A1	May 15, 2000	2000WO-AU00441	
MX2001011721A1	November 14, 2001	2001MX-0011721	
MX2001011721A1		WO 200070348	Based on

WO 200070348A1	May 15, 2000	2000WO-AU00441	
AU 200043862A	May 15, 2000	2000AU-0043862	
AU 200043862A		WO 200070348	Based on
BR 200010558A	May 15, 2000	2000BR-0010558	
BR 200010558A	May 15, 2000	2000WO-AU00441	
BR 200010558A		WO 200070348	Based on
EP 1183540A1	May 15, 2000	2000EP-0924979	
EP 1183540A1	May 15, 2000	2000WO-AU00441	
EP 1183540A1		WO 200070348	Based on
CN 1355885A	May 15, 2000	2000CN-0809065	
KR2002033093A	November 14, 2001	2001KR-0714533	
JP2002544520W	May 15, 2000	2000JP-0618732	
JP2002544520W	May 15, 2000	2000WO-AU00441	
JP2002544520W		WO 200070348	Based on
US20040157277A1	May 15, 2000	2000WO-AU00441	Cont of
US20040157277A1	March 8, 2002	2002US-0979594	Cont of
US20040157277A1	October 28, 2003	2003US-0695111	

INT-CL (IPC): C12 Q 1/02; C12 Q 1/68; G01 N 33/53; G01 N 33/554; G01 N 33/569; G01 N 33/574

ABSTRACTED-PUB-NO: WO 200070348A
BASIC-ABSTRACT:

NOVELTY - Diagnosis of gastric cancer or determining the risk of developing gastric cancer in patients with a Helicobacter infection using levels of IgG2 anti-Helicobacter pylori antibody, gamma -interferon (gamma -IFN) or interleukin-4 (IL-4) as markers.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(A) diagnosing gastric cancer and/or determining the risk of developing gastric cancer, in a subject with a Helicobacter infection, comprising:

(a) determining the level of IgG2 anti-Helicobacter pylori antibody, gamma -IFN, or IL-4 in the subject; and

(b) comparing this level with a predetermined control level, where a reduction in the level of the antibody, gamma -IFN or IL-4 in the subject, as compared to the control level, indicates the presence of gastric cancer and/or increased risk of developing gastric cancer;

(B) diagnosing gastric cancer and/or determining the risk of developing gastric cancer, in a subject with a Helicobacter infection, comprising:

(a) determining the frequency of cells which produce IgG2 anti-H. pylori antibody, gamma -IFN and/or IL-4 in the subject's blood or gastric mucosa; and

(b) comparing the frequency of such cells with predetermined control levels, where a reduction in the frequency of cells which produce the antibody or gamma -IFN, or an increase in the frequency of cells which produce IL-4, as compared to control levels, indicates the presence of gastric cancer and/or an increased risk of developing gastric cancer.

USE - The processes can be used for diagnosing gastric cancer or for determining the the risk of developing gastric cancer, in subjects suffering from Helicobacter infection. They can also be used for diagnosing, or determining the risk of developing pre-cancerous lesions such as metaplasia or dysplasia.

ADVANTAGE - The tests can be non-invasive and could be used as an alternative to endoscopy.

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TITLE: Diagnosis of gastric cancer, or assessment of the risk of developing it, using a largely non-invasive method which uses the level of e.g., gamma-interferon or interleukin-4, as a marker

Basic Abstract Text (1):
NOVELTY - Diagnosis of gastric cancer or determining the risk of developing gastric cancer in patients with a Helicobacter infection using levels of IgG2 anti-Helicobacter pylori antibody, gamma -interferon (gamma -IFN) or interleukin-4 (IL-4) as markers.

Basic Abstract Text (4):
(a) determining the level of IgG2 anti-Helicobacter pylori antibody, gamma -IFN, or IL-4 in the subject; and

Basic Abstract Text (7):
(a) determining the frequency of cells which produce IgG2 anti-H. pylori antibody, gamma -IFN and/or IL-4 in the subject's blood or gastric mucosa; and

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(54) Title: METHODS FOR MONITORING TREATMENT OF HELICOBACTER INFECTION AND FOR PREDICTING THE LIKELIHOOD OF SUCCESSFUL ERADICATION

(57) Abstract: The present invention relates to methods for monitoring treatment of Helicobacter infection and in particular to methods for monitoring eradication of *Helicobacter pylori* infection using immunoglobulin G2 (IgG2). The invention also relates to methods for predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection and in particular, to methods for predicting the likelihood of successful eradication including determining the levels of interleukin-4, interferon-γ and IgG in the subject to be, or being treated.

METHODS FOR MONITORING TREATMENT OF HELICOBACTER INFECTION AND FOR PREDICTING THE LIKELIHOOD OF SUCCESSFUL ERADICATION

TECHNICAL FIELD

The present invention relates to methods for monitoring treatment of Helicobacter infection and in particular to methods for monitoring eradication of *Helicobacter pylori* infection using immunoglobulin G2 (IgG2). The invention also relates to methods for predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection and in particular, to methods for predicting the likelihood of successful eradication including determining the levels of interleukin-4, interferon-γ and IgG in the subject to be, or being treated.

BACKGROUND ART

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Helicobacter pylori infection is now recognised as an essential pre-requisite for the development of gastric cancer. About 30% of the population become infected with this bacterium and commonly present with chronic gastritis. This may be complicated by gastric or duodenal ulceration, or may present as non-ulcer dyspepsia. A sizeable number of carriers are asymptomatic. However, in a small number of patients with *H. pylori,* their condition evolves through stages (including epithelial cell metaplasia and dysplasia) into neoplasia.

Current Management Practice of *H. pylori* Infection

Eradication of infection with antibiotics induces an 80-90% cure rate of peptic ulceration. A widely accepted treatment paradigm is based on detection of infection using antibody assays, followed by combination antibiotic therapy without prior endoscopic diagnosis. Endoscopy, before eradication therapy is generally accepted when 'danger' symptoms (eg, severe pain, bleeding) occur, or a significant risk of gastric cancer is present. However, endoscopy is a procedure which is associated with its own risks and is to be avoided if possible.

H. pylori initiates an IgG antibody response in saliva as well as serum. The serum IgG antibody is the basis of non-invasive diagnosis. Eradication of infection is followed by a very slow fall in serum antibody levels. There has been a study which suggests that IgG antibody levels at 6 months may be of value in assessing successful eradication. Saliva levels of IgG antibody however fall much quicker following eradication, with levels at 6 weeks regularly less than 80% of those prior to antibiotic therapy.

The concept that saliva IgG antibody levels may predict successful eradication, while attractive, proved not to be a practical proposition for monitoring of progress of treatment or eradication of Helicobacter because total IgG antibody levels were unstable to the extent that a viable test in clinical circumstances proved unreliable. At present, no non-invasive stable test exists which would allow successful monitoring of treatment designed to eradicate Helicobacter infection.

Further, in addition to monitoring eradication of *H. pylori* in individuals treated, it would be desirable to have a test which could be used prior to, or during treatment to determine the likelihood of successful eradication of *H. pylori*.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

SUMMARY OF THE INVENTION

According to a first aspect there is provided a method of monitoring eradication of Helicobacter infection in a subject treated for the infection, including:

a) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

b) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein a reduction in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates eradication of Helicobacter.

According to a second aspect there is provided a method of monitoring efficacy of treatment of Helicobacter infection in a subject treated for the infection, including:

a) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

b) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein a reduction in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates efficacious treatment of Helicobacter.

According to a third aspect there is provided a method of monitoring relapse or reinfection with Helicobacter in a subject treated for infection with Helicobacter, including:

a) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

b) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein an increase in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates relapse or reinfection with Helicobacter.

According to a fourth aspect there is provided a method of detecting unresponsiveness of a subject to treatment of Helicobacter infection, including:

a) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

b) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein lack of change in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates lack of response to treatment.

According to a sixth aspect there is provided a kit for monitoring treatment of Helicobacter infection, including,

a) Helicobacter antigen

b) reagent for determining IgG2 subclass antibody.

Preferably, the IgG2 anti-*H. pylori* antibody is detected by a near-subject assay. The assay may, however, also be a laboratory-based test. Preferably, the assay is an antibody assay although it will be understood that other known methods of measurement can also be effectively used. Most preferably, the assay is an immunoassay such as ELISA, RIA or a similar assay format.

Control levels of IgG2 anti-*H. pylori* antibody can be established in samples of saliva obtained from normal individuals, ie. those not having an established *H. pylori* infection. It is preferred however that control levels of IgG2 be determined in subject's own saliva prior to commencement of treatment for infection or, if monitoring relapse or reinfection, the levels of salivary IgG2 following successful eradication of Helicobacter.

According to a seventh aspect, the present invention provides a method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination of IL-4 level in a sample from the subject;

(ii) comparison of the IL-4 level with a predetermined control or standard IL-4 level,

(iii) wherein a level of IL-4 in the sample from the subject above the control or standard IL-4 level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard IL-4 level is predictive of the likelihood of eradication failure.

Preferably, the sample is a blood sample.

Preferably, the IL-4 is detected by an immunoassay and more preferably, it is determined by ELISA.

The skilled addressee will readily be able to identify a suitable control or standard IL-4 level. For example, the control or standard level of IL-4 may be established from analysis of samples obtained from subjects not infected by *H. pylori* and/or subjects having successfully eradicated *H. pylori* and/or subjects infected by *H. pylori.*

According to an eighth aspect, the present invention provides a method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination of interferon-γ (INF-γ) level in a sample from the subject;

(ii) comparison of the INF-γ level with a predetermined control or standard INF-γ level,

(iii) wherein a level of INF-γ in the sample from the subject below the control or standard INF-γ level is predictive of the likelihood of successful eradication and a level of IFN- γ above the control or standard level is predictive of the likelihood of eradication failure.

Preferably, the INF-γ level is determined in a blood sample.

Preferably, the INF-γ level is detected by an immunoassay and preferably the assay is ELISA.

The skilled addressee will readily be able to establish a suitable control or standard. For example, the control or standard level of IFN- γ may be established from analysis of samples obtained from subjects not infected by *H. pylori* and/or subjects having successfully eradicated *H. pylori* and/or subjects infected by *H. pylori.*

According to a ninth aspect, the present invention provides a method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination of immunoglobulin G (IgG) level in a sample from the subject;

(ii) comparison of the IgG level with a predetermined control or standard IgG level,

(iii) wherein a level of IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

Preferably, the IgG level is determined in a serum sample and, more preferably, the sample is a saliva sample.

The skilled addressee will readily be able to establish a suitable control or standard level of IgG. For example, the control or standard level of IgG may be established from analysis of samples obtained from subjects not infected by *H. pylori* and/or subjects having successfully eradicated *H. pylori* and/or subjects infected by *H. pylori*.

According to a tenth aspect, the present invention provides a method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination a combination of IL-4 and/or INF-γ and/or IgG levels in a sample from the subject;

(ii) comparison of the IL-4 and/or INF-γ and/or IgG levels with a predetermined control or standard IL-4 and/or INF-γ and/or IgG level respectively,

wherein a level of IL-4 in the sample from the subject above the control or standard level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard level is predictive of the likelihood of eradication failure, and

wherein a level of INF-γ in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IFN- γ above the control or standard level is predictive of the likelihood of eradication failure, and

wherein a level of IgG in the sample from the subject below the control or standard level is

predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Stability of salivary IgG2 anti-*Helicobacter pylori* antibody.

Figure 2 Salivary IgG (panel A) and IgG2 (panel B) anti-H. pylori antibody before and after eradication of *H. pylori*.

Figure 3 Salivary IgG (panel A) and IgG2 (panel B) anti- *H. pylori* antibody in subject with and without *H. pylori* infection.

Figure 4 Correlation between IL-4 production in whole blood and gastric tissue cultures. Whole blood cultures or gastric antrum biopsy cultures were incubated for 24 hours at 37°C, after which time the levels of IL-4 were measured by ELISA capture assay. The results shown a correlation between mucosal and whole blood IL-4 ($p<0.001$).

Figure 5 Levels of IL-4 in whole blood culture stimulated with *H. pylori* AGE antigen. Peripheral blood obtained from subjects with or without *H. pylori* infection, or with eradication failure was added to equal volume of AIM-V culture medium containing graded concentrations of *H. pylori* AGE antigen as indicated. After 24 hours of culture, levels of IL-4 were measured by ELISA capture assay. Results shown are the mean ± standard error of the mean. *: $p<0,05$: compared with *H. pylori*-eradicated subjects; ¶: $p< 0,01$ and $p<0.05$ compared with the values from subjects with *H. pylori*-eradicated and *H. pylori*-positive, respectively.

Figure 6 IFN-γ production in response to *H. pylori* acid-glycine extract stimulation in whole blood. Peripheral blood was collected from individual subject and cultured in the presence of graded concentration of *H. pylori* AGE antigen for 24 hours. Culture supernatants were collected and assayed for IFN-γ by ELISA. Results shown were mean ± standard error of the mean. NS: Not Significant.

Figure 7 Levels of specific *H. pylori* IgG antibody in serum and saliva. Serum and saliva samples were collected from individual subjects. Levels of specific *H. pylori* IgG were measured by ELISA. Results shown were mean ± standard error of the mean. *: $p<0.05$ compared with mean from *H. pylori*-positive group; NS: Not Significant.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

It has surprisingly been found that salivary IgG2 anti-*H. pylori* antibody is stable and allows a reliable test to be developed for monitoring progress of treatment and/or eradication of *Helicobacter pylori* infection in a subject undergoing treatment.

It was previously known that IgG anti-*H. pylori* antibody levels in blood and gastric mucosa can be used as an indicator of *H. pylori* status. There has been an attempt to use IgG anti- *H. pylori* antibody in saliva for a similar purpose but it proved to be unstable in such a sample. From the following examples it will be understood that while IgG anti-*H. pylori* may be useful as a general indicator of *H. pylori* status, it is the measurement of the IgG2 subclass anti- *H. pylori* antibody which allows a stable treatment monitoring test to be developed.

It has further surprisingly been found that IL-4 levels can be used as a predictor of successful eradication of *H. pylori*. It is envisaged that an IL-4 test could be used prior to, or during the treatment of *H. pylori* infection in order to predict the likelihood of eradication.

Techniques for measurement of antibodies and IL-4 in human samples are well-known in the art and protocols and reagents are readily available. Examples of some of the techniques used are indicated below as an illustration of how some measurements may be performed.

Unless indicated otherwise, standard techniques which can be ascertained from standard texts and laboratory manuals may be employed.

The invention will now be described in more detail with reference to non-limiting examples.

EXAMPLES

Example 1 Determination of antibody levels in saliva samples

Sample Collection

Saliva samples were collected from 4 patients infected with *H. pylori* who were treated with eradication triple therapy comprised of amoxycillin, omeprazole and clarithromycin for seven days. Samples were taken before treatment and after 10 days of eradication therapy.

H. pylori antigen preparation

H. pylori NCTC 11637 strain was used for *H.pylori* antigen preparation according to modified methods described by Goodwin (#208). Protein concentration in the extract was measured using a bio-rad kit (Bio-rad laboratories Australia). Aliquots were stored at -70°C.

Antibody detection by ELISA

For saliva anti-*H. pylori* antibody detection, wells of a 96-well flat-bottomed microtiter Polysorb plate (Nunc, Denmark) were coated with 7 μg/mL of *H. pylori* antigen at 4°C overnight. After washing and blocking the plates with 5% skim milk (Diploma, Australia) in PBS-Tween 20, saliva samples at 1:2 dilution with 2% PEG 6000 were added to individual wells in triplicate. After incubation, the wells were washed and horseradish peroxidase conjugated-sheep anti-IgG or anti-IgG2 (Silenus, Australia) at 1:2000 dilution was added to each well. Following a further incubation, the plates were washed and then tetramethyl benzidine (TMB) substrate (Sigma, USA) was added to each well. The reaction was stopped using 1 mol/L H_2SO_4 and the absorbance was read at 450 nm in an ELISA plate reader (Bio-Rad 450, Japan). The results were expressed as ELISA INDEX being the mean OD_{450} of a given saliva sample divided by the mean OD_{450} of the calibrating sample. Positive and

negative quality control samples were included in each run to control for intra- and inter-assay variation.

Saliva samples were obtained from 5 subjects infected with *H. pylori*. The samples were tested for IgG2 and total IgG anti-*H. pylori* antibody by the ELISA assay either fresh or after storage for up to 12 months. The results show that IgG2 antibody levels were more stable than IgG antibody levels (Figure 1). Hence, IgG2 antibody is a reliable and a sensitive indicator of infection status due to its stability during storage and assay.

Example 2 Anti-*H. pylori* antibody levels in saliva from patients undergoing eradication therapy.

Saliva samples from subjects undergoing antibiotic eradication therapy were tested for anti-*H. pylori* antibody using the immunoassay method described in Example 1.

IgG and IgG2 antibody was measured before and after treatment with antibiotics. Ten days after treatment IgG2 antibody levels fell quicker than total IgG antibody levels (Figure 2A and 2B).

In a separate study it was shown that saliva from subjects with *H. pylori* infection have markedly elevated levels of IgG2 (Figure 3A) when compared to subjects without infection (Figure 3B). Subjects who failed to ultimately eradicate the infection did not demonstrate a significant drop in the level of IgG2 anti-*H. pylori* antibody.

Example 3 - Interleukin-4/IFN-γ and IgG Studies

Subjects

Fifty-two subjects referred for investigation of dyspepsia, and 11 subjects with persistent *H. pylori* infection following one or more courses of antibiotics, were recruited for this study. Subjects with dyspepsia had not taken antibiotics within three months of the study. The study was approved by the Ethics Committee of the Centre for Digestive Diseases, Sydney,

Australia. Informed consent was obtained from all patients. Multiple biopsy specimens were obtained during upper gastrointestinal endoscopy from the antrum and the body of the stomach to be used for tissue culture, histology and a urease test (CLO test, Delta West, WA, Australia). Blood samples were incubated at 37°C within 2 hours of collection. Serum was stored at -70°C for *H. pylori* specific antibody.

Saliva sample collection

Saliva samples were collected before the endoscopy procedure. Samples were centrifuged at 1000 x *g* for 10 minutes at 4°C, and aliquots were stored at -70°C.

Biopsy culture

Gastric biopsy tissues were weighted and cultured at a ratio of 50 μL serum-free AIM-V medium (Life Technology, Australia) per mg tissue (wet weight) for 24 hours. The culture supernatants were collected and centrifuged. Aliquots were stored at -70°C until assay.

H. pylori antigen preparation

H. pylori antigens from the NCTC 11637 strain were prepared by acid-glycine extraction (AGE) according to the method described by Goldwin et al (*J Infect Dis* 1987; 155:488-94). *H. pylori* AGE was used for cell culture and specific antibody measurement.

ELISA capture assay for IL-4 in whole blood culture

Cytokine levels in whole blood culture were measured following the method described previously (Ren et al, *Helicobacter* 2000; 5:135-41). Briefly, 150 μL of heparinized whole blood was added in triplicate to wells of a 96-well microtitre flat-bottomed plate pre-coated with mouse polyclonal anti-human IL-4 antibody (Endogen, MA, USA). An equal volume of AIM-V medium containing *H. pylori* AGE at either 0, 1 or 10 μg/mL was also loaded to wells. The cultures were incubated at 37°C with 5% CO_2 for 24 hours, after which time supernatants were collected for interferon-γ (IFN-γ) assay. The amount of 'captured' IL-4 was measured by ELISA as following. Briefly, after washing the plates, biotinylated mouse

monoclonal anti-human IL-4 antibody (Endogen, MA, USA) was added (0.5 μg/mL) to wells and incubated for 90 minutes at room temperature. The plates were then washed and incubated for a further 30 minutes at room temperature with streptavidin-conjugated horse-radish peroxidase (Selinus, Australia) at a 1:400 dilution. The plates were thoroughly washed with washing buffer and finally incubated for 10 minutes at room temperature with 3,3'-5,5' tetramethyl benzidine (TMB, Sigma-Aldrich, USA) substrate. The reaction was stopped using 1 mol/L H_2SO_4 and optical density at 450 nm (OD 450nm) was measured in an ELISA plate reader (Bio-Rad 450, Japan). Standard IL-4 (Endogen, MA, USA) was applied for each plate to control plate to plate variation. The limits of sensitivity for IL-4 was 9.4 pg/mL. The amount of IL-4 in samples was determined using a Softmax program (Version 2.3 FPU, USA).

IFN-γ ELISA assay

Wells of a 96-well flat-bottomed microtitre plate (Nunc, Denmark) were coated with mouse anti-human IFN-γ monoclonal antibody (Endogen, MA, USA) at 2 μg/mL overnight at 4°C. After washing and blocking, supernatants from whole blood culture or IFN-γ standards (Endogen, MA, USA) were added in duplicate, and incubated for 90 minutes. The plates were washed and biotinylated mouse monoclonal anti-human IFN-?γ antibody (Endogen, MA, USA) was added (0.25 μg/mL). After 90 minutes incubation, the wells were washed and streptavidin-conjugated horse-radish peroxidase (Selinus, Australia) was applied at a 1:2000 dilution. The plates were washed and TMB chromagen (Sigma-Aldrich, USA) was added to each well. The absorbance was read at 450 nm in an ELISA plate reader (Bio-Rad 450, Japan). The limits of sensitivity for IFN-γ was 9.4 pg/mL. The amount of IFN-γ in samples was determined using a Softmax program (Version 2.3 FPU, USA).

Detection of *H. pylori* antibody

Wells of a 96-well flat-bottomed microtitre plate were coated with *H. pylori* AGE at 5

μg/mL at 4°C overnight. After washing and blocking, serum samples at 1:3000 dilution and saliva sample at 1:4 dilution were added to wells in triplicate. Horse-radish peroxidase conjugated-sheep anti-IgG (Selinus, Australia) was applied at 1:2000 dilution. Tetramethyl Benzidine (TMB) substrate (Sigma-Aldrich, USA) was used for colour development. The absorbance was read at 450 nm in an ELISA plate reader (Bio-Rad, 450, Japan). The results were expressed as ELISA Units against a reference standard of pooled positive sera. Intra- and inter-assay variation was less than 10%.

Statistical analysis

Data were expressed as mean ± standard error (SE). Correlation Z test was used to test for a correlation between mucosal and blood cytokine production. Differences of means among patient groups were analysed by ANOVA. All statistical analysis were performed by using a StatView 4.5 software program (Abacus Concepts, California, USA). Significant difference was considered when p value was less than 0.05.

RESULTS

Subjects were divided into four groups according to *H. pylori* infection status and results of antibiotic treatment. There were 23 *H. pylori*-negative subjects; 20 *H. pylori*-positive subjects; 9 subjects with successful *H. pylori* eradication confirmed by histology or C^{14} breath test at 6-8 weeks after eradication therapy; and 11 subjects with *H. pylori* resistance following antibiotic therapy. Details of diagnosis and therapeutic regimens in subjects with eradication failure are shown in Table 1.

Comparison of blood and mucosal IL-4 response

To determine whether there is a correlation between blood and mucosal cytokine responses to *H. pylori* infection, levels of IL-4 production in whole blood cultures stimulated or unstimulated with *H pylori* antigens, were compared with levels in gastric mucosa cultures (Fig. 1) (data from antigen stimulated cultures not shown). The results from *H pylori* positive

(n=6) and negative subjects (n=11) and subjects with failed eradication (n=8) showed that IL-4 production in whole blood cultures (stimulated or unstimulated) correlated with that in gastric mucosa (r^2=0.549, p<0.001).

IL-4 and IFN-γ production in whole blood culture

Significantly lower levels of IL-4 were detected in whole blood stimulated or unstimulated with *H. pylori* AGE from subjects with eradication failure compared with subjects in whom *H. pylori* was successfully eradicated (p<0.05, 0 and 1.0 μg/mL *H. pylori* AGE; p<0.01, 10 μg/mL *H. pylori* AGE) or in subjects with untreated infection (p<0.05, 10 μg/mL *H. pylori* AGE) (Fig 2). IL-4 levels were similar in non-infected and infected subjects, and were not significantly different when compared to subjects with successful eradication (though there was a trend towards increased levels following eradication). Although there was no statistically significant difference in the levels of IFN-γ between the different groups, lower levels were detected in subjects with successful *H. pylori* eradication (Fig. 3). Low levels of IL-4 secretion were seen in most subjects with ongoing infection with resistant *H.pylori*, irrespective of the number of courses of therapy (Table 2).

Anti-*H. pylori* IgG levels in serum and saliva

Both serum and saliva IgG antibody levels were significantly lower in non-infected subjects (p<0.05) and in subjects at 6-8 weeks after eradication therapy (p<0.05) than in subjects who were positive for *H. pylori*. For both saliva and serum antibody, a trend towards lower levels of antibody in those failing to eradicate infection was seen, but this was short of statistical significance (Fig. 4).

Table 1: Clinical Characterisation of Subjects with Failed Antibiotic Therapy

No.	Age (years)	Diagnosis	Treatment Regimens Used	Number of Antibiotic Courses	Duration (months)
1	40	Hp-induced gastritis	metronidazole/amoxicillin/bismuth/ranitidine HCl	1	24
			clarithromycin/metronidazole/lansoprazole/amoxicillin	2	
2	58	Hp-induced gastritis	Losec HP7	1	12
3	55	Oesophagitis and Hp-induced gastritis	Klacid HP7	1	>3yrs
			Helidac/ranitidine HCl	1	
			lansoprazole	1	
4	47	Hp-induced gastritis	Losec HP7	2	20
5	37	Hp-induced gastritis	metronidazole	1	5
			Losec HP7	3	
6	45	Hp-induced gastritis	Losec HP7/ranitidine HCl	3	28

No.	Age (years)	Diagnosis	Treatment Regimens Used	Number of Antibiotic Courses	Duration (months)
7	27	Hp-induced gastritis	Losec HP7	3	6
			clarithromycin/tetracycline/metronidazole/lansoprazole	1	
8	33	Hp-induced gastritis and duodenal ulcer disease	Helidac	2	>3yrs
9	26	Hp-induced gastritis	Losec HP7	2	10
10	47	Hp-induced gastritis	Losec HP7	3	>3yrs
11	73	Oesophagitis, Hp-induced gastritis and duodenal ulcer disease	Losec HP7	3	>3yrs

Hp= *Helicobacter pylori;* Helidac= bismuth/metronidazole/tetracycline; Klacid HP7= omeprazole/amoxicillin/clarithromycin;

Losec HP7= omeprazole/amoxicillin/clarithromycin

Table 2: IL-4 and *H. pylori* Antibody IgG in Subjects with Failure Eradication

Times of failure	No. Subjects	IL-4 levels (pg/mL)*			H. pylori Antibody IgG*	
		H. pylori antigen (0 μg/mL)	*H. pylori* antigen (1 μg/mL)	*H. pylori* antigen (10 μg/mL)	Serum (ELISA Unit)	Saliva (ELISA Unit)
One	1	20.76	28.21	44.20	214	116.3
Two	3	40.49 ± 29.36	54.07 ± 43.14	65.22 ± 45.86	224 ± 101.58	1000.2 ± 866.5
Three	5	45.16 ± 36.16	53.34 ± 44.34	55.63 ± 44.19	410.95 ± 167.29	418.9 ± 151.96
Four	2	18.82 ± 9.82	22.56 ± 13.58	12.60 ± 3.6	1453.6 ± 1244.4	523.7 ± 235.3

* Standard error of mean (SEM).

The skilled addressee will understand that, in light of the above, IL-4, INF-γ and IgG can be used to predict the likelihood of successful eradication of Helicobacter infection before or during treatment of the infection. As a corollary, it will be clear that the method can also be used to identify subjects unlikely to respond to treatment for Helicobacter infection.

Although the invention has been described with reference to specific examples, it will be appreciated by those skilled in the art that the invention may be embodied in many other forms without departing from the spirit or intent of the inventive concept.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of monitoring eradication of Helicobacter infection in a subject treated for the infection, including:

   i) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

   ii) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein a reduction in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates eradication of Helicobacter.

2. A method of monitoring efficacy of treatment of Helicobacter infection in a subject treated for the infection, including:

   i) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

   ii) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein a reduction in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates efficacious treatment of Helicobacter.

3. A method of monitoring relapse or reinfection with Helicobacter in a subject treated for infection with Helicobacter, including:

   i) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

ii) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein an increase in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates relapse or reinfection with Helicobacter.

4. A method of detecting unresponsiveness of a subject to treatment of Helicobacter infection, including:

(i) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

(ii) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein lack of change in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates lack of response to treatment.

5. A method according to any one of claims 1 to 4, wherein the IgG2 anti-*H. pylori* antibody is detected by an immunoassay.

6. A method according to claim 5, wherein the assay is ELISA.

7. A method according to any one of claims 1 to 6, wherein the control levels of IgG2 anti-*H. pylori* antibody is established in samples of saliva obtained from subjects not infected by *H. pylori.*

8. A method according to any one of claims 1 to 6, wherein the control levels of IgG2 anti-*H. pylori* antibody are determined in subject's own saliva sample.

9. A kit for monitoring treatment of Helicobacter infection, including,

(i) Helicobacter antigen

(ii) reagent for determining IgG2 subclass antibody.

10. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination of IL-4 level in a sample from the subject;

(ii) comparison of the IL-4 level with a predetermined control or standard IL-4 level,

(iii) wherein a level of IL-4 in the sample from the subject above the control or standard IL-4 level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard IL-4 level is predictive of the likelihood of eradication failure.

11. A method according to claim 10 wherein the sample is a blood sample.

12. A method according to claim 10 or claim 11, wherein the IL-4 is detected by an immunoassay.

13. A method according to claim 12, wherein the assay is ELISA.

14. A method according to any one of claims 10 to 13, wherein the control or standard level of IL-4 is established from analysis of samples obtained from subjects not infected by *H. pylori* and/or subjects having successfully eradicated *H. pylori* and/or subjects infected by *H. pylori*.

15. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination of interferon-γ (INF-γ) level in a sample from the subject;

(ii) comparison of the INF-γ level with a predetermined control or standard INF-γ level,

(iii) wherein a level of INF-γ in the sample from the subject below the control or standard INF-γ level is predictive of the likelihood of successful eradication and a level of IFN- γ above the control or standard level is predictive of the likelihood of eradication failure.

16. A method according to claim 15 wherein the sample is a blood sample.

17. A method according to claim 15 or claim 16, wherein the IFN- γ level is detected by an immunoassay.

18. A method according to claim 17, wherein the assay is ELISA.

19. A method according to any one of claims 15 to 18, wherein the control or standard level of IFN- γ is established from analysis of samples obtained from subjects not infected by *H. pylori* and/or subjects having successfully eradicated *H. pylori* and/or subjects infected by *H. pylori.*

20. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination of immunoglobulin G (IgG) level in a sample from the subject;

(ii) comparison of the IgG level with a predetermined control or standard IgG level,

(iii) wherein a level of IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

21. A method according to claim 20 wherein the sample is a serum sample.

22. A method according to claim 20 wherein the sample is a saliva sample.

23. A method according to any one of claims 20 to 22, wherein the control or standard level of IgG is established from analysis of samples obtained from subjects not infected by *H. pylori* and/or subjects having successfully eradicated *H. pylori* and/or subjects infected by *H. pylori.*

24. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination a combination of IL-4 and/or INF-γ and/or IgG levels in a sample from the subject;

(ii) comparison of the IL-4 and/or INF-γ and/or IgG levels with a predetermined control or standard IL-4 and/or INF-γ and/or IgG level respectively, wherein a level of IL-4 in the sample from the subject above the control or standard level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard level is predictive of the likelihood of eradication failure, and

wherein a level of INF-γ in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IFN- γ above the control or standard level is predictive of the likelihood of eradication failure, and

wherein a level of IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

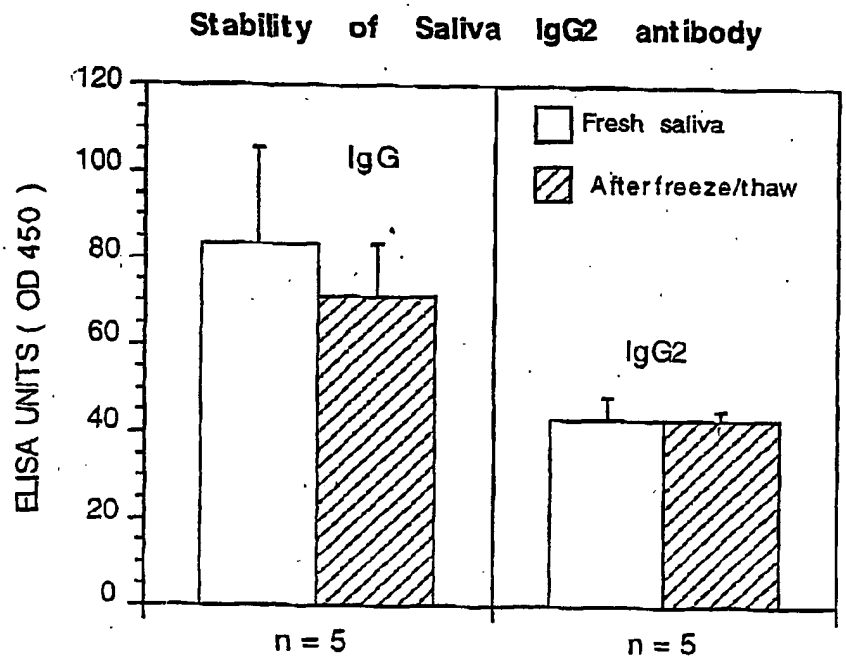
Stability of Saliva IgG2 antibody
ELISA UNITS (OD 450)
120
100
80
60
40
20
0
IgG
IgG2
Fresh saliva
After freeze/thaw
n = 5
n = 5

Figure 1

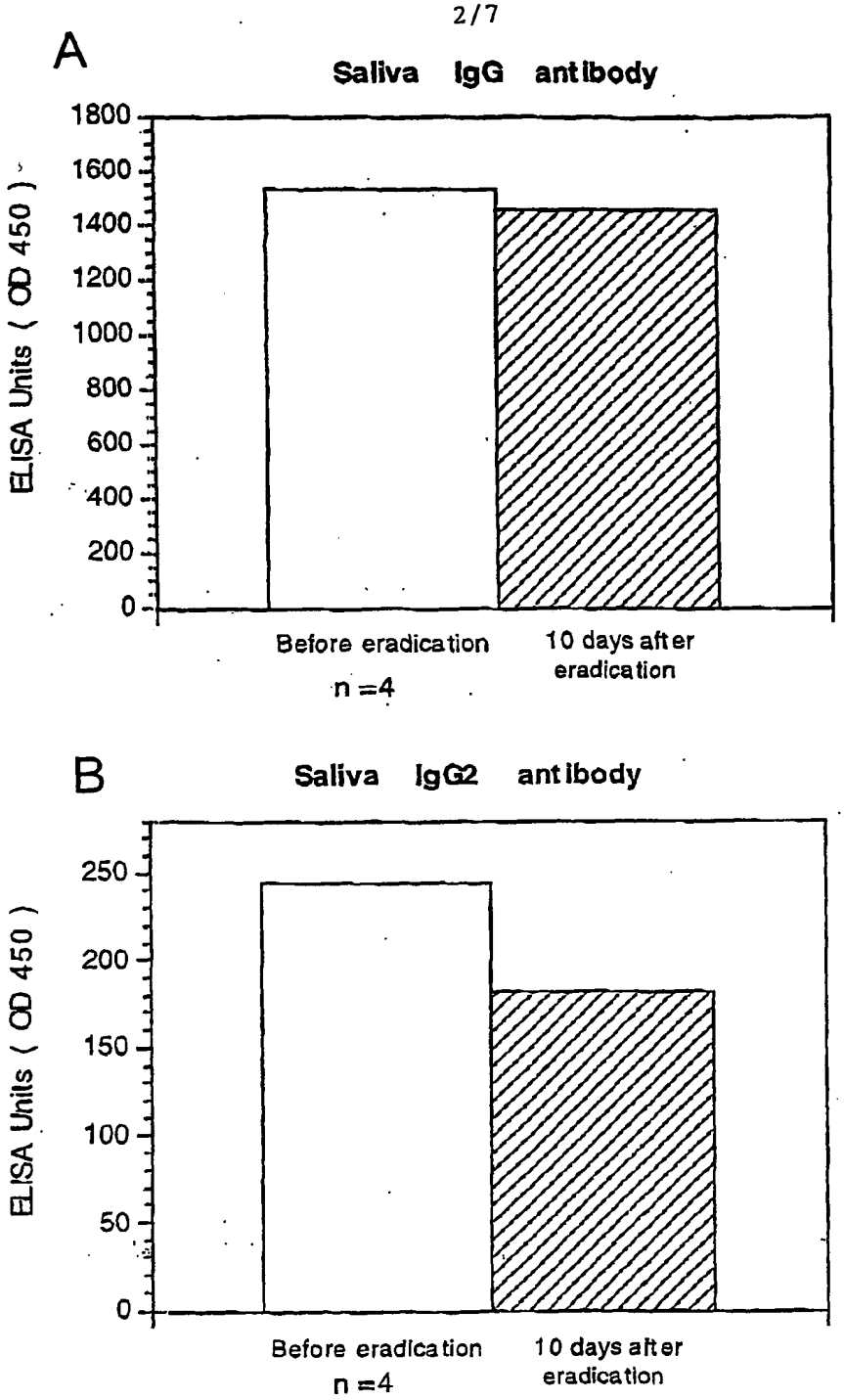
A
Saliva IgG antibody
ELISA Units (OD 450)
1800
1600
1400
1200
1000
800
600
400
200
0
Before eradication
n =4
10 days after eradication
B
Saliva IgG2 antibody
ELISA Units (OD 450)
250
200
150
100
50
0
Before eradication
n =4
10 days after eradication

Figure 2

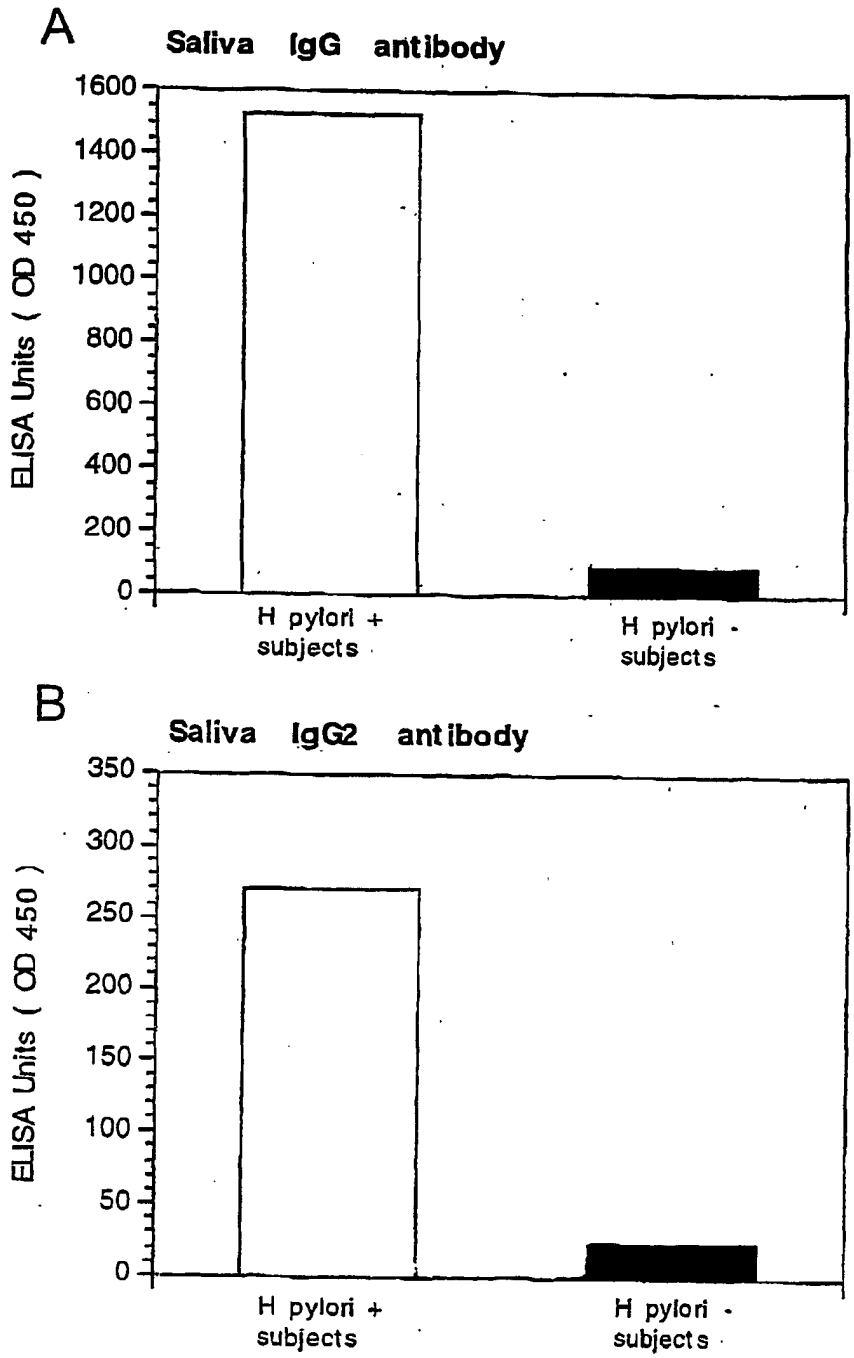
A
Saliva IgG antibody
ELISA Units (OD 450)
1600
1400
1200
1000
800
600
400
200
0
H pylori + subjects
H pylori - subjects
B
Saliva IgG2 antibody
ELISA Units (OD 450)
350
300
250
200
150
100
50
0
H pylori + subjects
H pylori - subjects

Figure 3

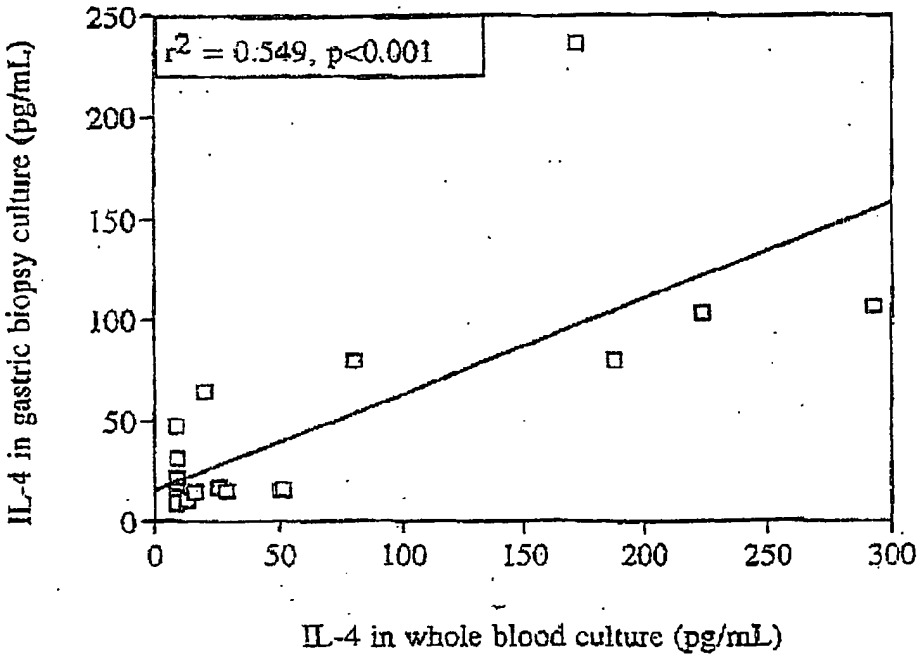
$r^2 = 0.549$, $p<0.001$
IL-4 in gastric biopsy culture (pg/mL)
IL-4 in whole blood culture (pg/mL)
0
50
100
150
200
250
300

FIGURE 4

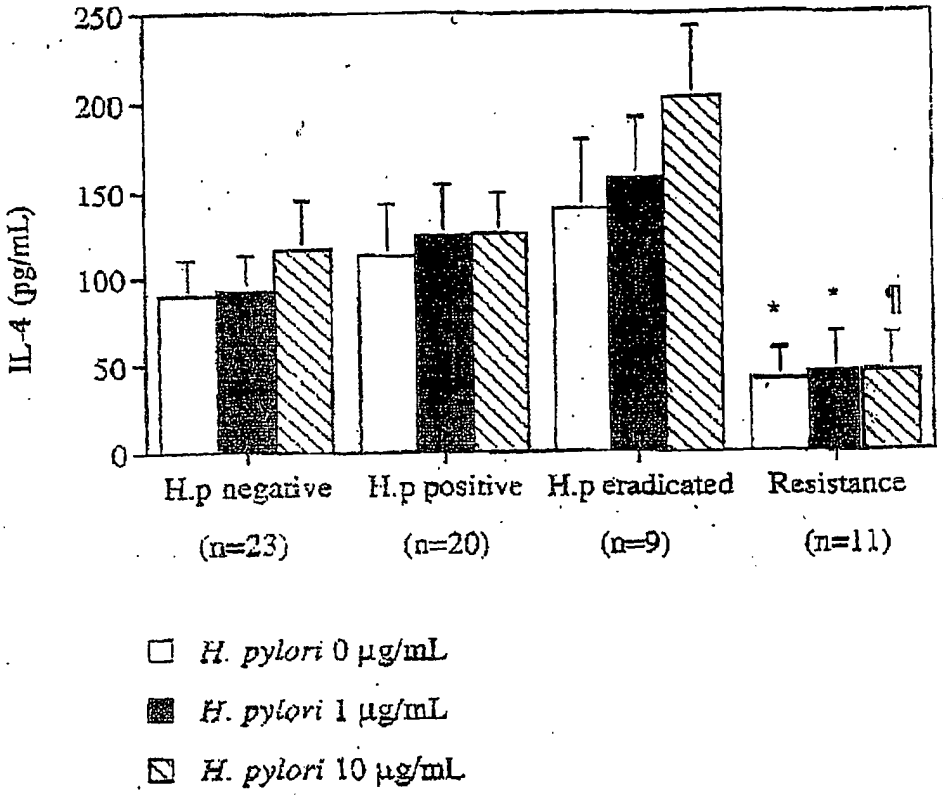
250
200
150
100
50
0
IL-4 (pg/mL)
H.p negative
(n=23)
H.p positive
(n=20)
H.p eradicated
(n=9)
Resistance
(n=11)
*
*
¶
H. pylori 0 µg/mL
H. pylori 1 µg/mL
H. pylori 10 µg/mL

FIGURE 5

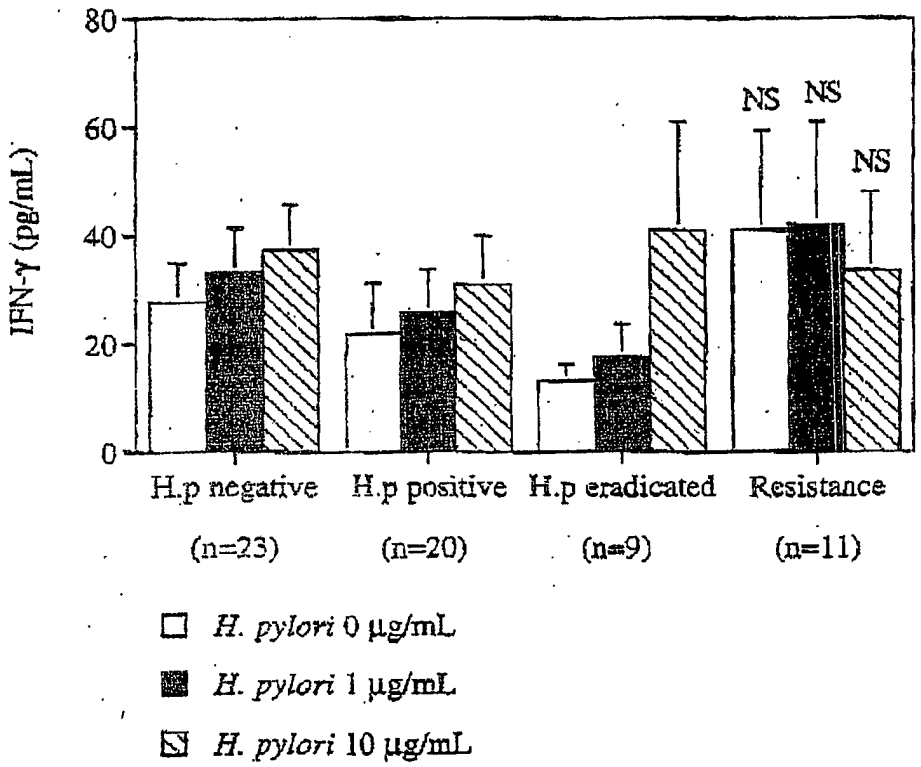
80
60
40
20
0
IFN-γ (pg/mL)
NS
NS
NS
H.p negative
H.p positive
H.p eradicated
Resistance
(n=23)
(n=20)
(n=9)
(n=11)
H. pylori 0 µg/mL
H. pylori 1 µg/mL
H. pylori 10 µg/mL

FIGURE 6

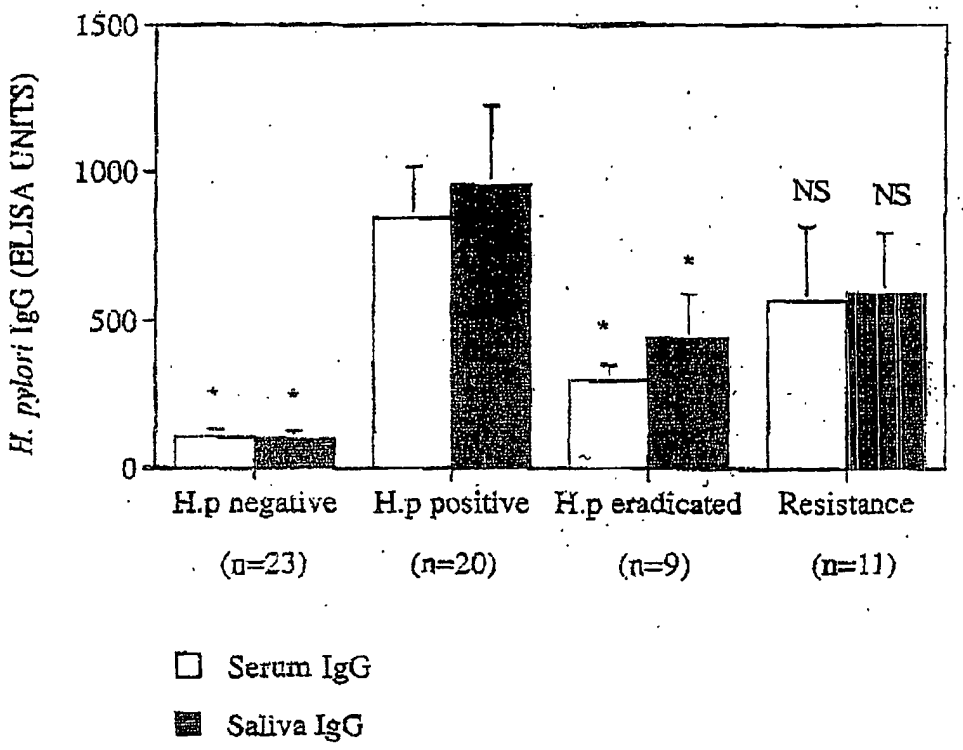
1500
1000
500
0
H. pylori IgG (ELISA UNITS)
NS
NS
*
*
*
*
H.p negative
(n=23)
H.p positive
(n=20)
H.p eradicated
(n=9)
Resistance
(n=11)
Serum IgG
Saliva IgG

FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00795

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: G01N 33/53, G01N 33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl. [7]: G01N 33/53, G01N 33/569

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, WPAT, JAPIO, esp@ce, PubMed, Delphion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Australian patent 714222 by Cortecs Ltd open to public inspection in Australia 4 September 1996 (WO 96/25430 published 22 August 1996), which relates to a method of diagnosing H. pylori by detecting antibodies in saliva using antigens, see especially claims 8, 9 - 11, and related kit for use in diagnosis. See pages 1, 2, 4 - 6, and examples.	1-9
X	WO 98/32768 by Cortecs Ltd published 30 July 1998, which also relates to a method of diagnosing H. pylori by detecting antibodies in saliva using antigens, see especially claims 11 - 13, 15, 21 and related kit for use in diagnosis in the examples and claim 16. See pages 1,2, 4,5, 12, 17 and examples.	1-9
X	WO 00/29432 by Cortecs Ltd published 11 November 1999 also relates to a method of diagnosing H. pylori by detecting antibodies in saliva using antigens, see especially claims 11, 12, 14, 15, 19 and related kit for use in diagnosis in the examples and claim 20 - 22. See page 10 and examples.	1-9

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 August 2001	31 AUGUST 2001
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer ANTHEA HARVIE Telephone No : (02) 6283 2552

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU01/00795

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	9832768	AU	58715/98	EP	975663

END OF ANNEX

Hit List

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Search Results - Record(s) 1 through 32 of 32 returned.

☐ 1. Document ID: US 20040236088 A1

Using default format because multiple data bases are involved.

L9: Entry 1 of 32 File: PGPB Nov 25, 2004

PGPUB-DOCUMENT-NUMBER: 20040236088
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040236088 A1

TITLE: Novel polypeptide analogs and fusions and their methods of use

PUBLICATION-DATE: November 25, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Heuer, Josef Georg	Indianapolis	IN	US	
Na, Songqing	Carmel	IN	US	
Okragly, Angela Jeannine	Indianapolis	IN	US	
Ou, Weijia	Fishers	IN	US	

US-CL-CURRENT: 536/23.2; 424/94.6, 435/320.1, 435/325, 435/69.1, 530/350, 530/388.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. D

☐ 2. Document ID: US 20040208863 A1

L9: Entry 2 of 32 File: PGPB Oct 21, 2004

DOCUMENT-IDENTIFIER: US 20040208863 A1
TITLE: Anti-inflammatory activity from lactic acid bacteria

Detail Description Paragraph:
[0126] To further understand the implications of TNF-.alpha. inhibition by LGG on the cytokine network of the innate immune response, we evaluated cytokine profiles of LPS-stimulated macrophages in the presence or absence of LGG-cm. Bioassays were performed and cytokines quantitated by the Luminex LabMAP 100.TM. System (Martins et al., 2002). Interleukin-1.beta. (IL-1.beta.), IL-10, IL-12 and TNF-.alpha., but not granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-6 (IL-6) and interferon-gamma (IFN-.gamma.), were detected in culture supernatants of LPS-stimulated macrophages. Levels of IL-1 .beta. and IL-10 in LGG-treated-LPS-

stimulated macrophages were comparable to quantities produced by LPS-stimulated cells. A seven-fold reduction was observed in TNF-.alpha. levels in LGG-treated LPS-LPS-stimulated cells compared to LPS alone, similar to ELISA data. Interestingly, the levels of IL-10 were unaffected whether macrophages were exposed to LPS alone or or co-incubated with LGG-cm. LGG-treated macrophages had diminished TNF-.alpha./IL-10 ratios compared to LPS alone (FIG. 6) indicating a net immunomodulatory effect. Since Gram-negative bacterial-derived products stimulate naive macrophages, we wanted to establish whether LGG could prevent TNF-.alpha. production induced by E. coli or pathogenic helicobacters. In our assay, conditioned media of Gram-negative bacteria such as E. coli, H. pylori or H. hepaticus, are capable of inducing TNF-.alpha. secretion by macrophages. However, neither H. pylori- or H. hepaticus-derived P/CAMPs present in conditioned media are as potent as E. coli-derived P/CAMPs in stimulating TNF-.alpha. secretion in macrophages. Intragenus comparison of macrophage activation shows that H. pylori-conditioned media elicits about 900 .rho.g/ml TNF-.alpha. while H. hepaticus produces approximately half of H. pylori-induced levels. In the presence of LGG-cm, TNF-.alpha. induction is significantly inhibited indicating antagonism of LGG-derived immunomodulins versus Helicobacter-derived immunostimulatory factors ($p<0.01$). It is interesting to note that induction by E. coli is not affected by the addition of LGG-cm. LGG may inhibit TNF-.alpha. only when LPS (or an immunostimulatory P/CAMP) of a given nature or particular threshold concentration is present (FIG. 7).

☐ 3. Document ID: US 20040192612 A1

L9: Entry 3 of 32 File: PGPB Sep 30, 2004

DOCUMENT-IDENTIFIER: US 20040192612 A1
TITLE: Caspase inhibitors and uses thereof

Summary of Invention Paragraph:
[0176] The above-described compositions are particularly useful in therapeutic applications relating to an IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, a proliferative disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, retinal disorders, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, asthma, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Grave's disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis, scarring, graft vs host disease, organ transplant rejection, organ apoptosis after burn injury, osteoporosis, leukemias and related disorders, myelodysplastic syndrome, multiple myeloma-related bone disorder, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, multiple myeloma, hemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia, epilepsy, myocardial ischemia, acute and chronic heart disease, myocardial infarction, congestive heart failure, atherosclerosis, coronary artery bypass graft, spinal muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, ulcerative colitis, traumatic brain injury, spinal cord injury, hepatitis-B, hepatitis-C, hepatitis-G, yellow fever, dengue

fever, Japanese encephalitis, various forms of liver disease, renal disease, polycystic kidney disease, H. pylori-associated gastric and duodenal ulcer disease, HIV infection, tuberculosis, and meningitis. The compounds and compositions are also useful in treating complications associated with coronary artery bypass grafts. The compounds and compositions are also useful for decreasing IGIF or IFN-.gamma. production. The compounds and compositions are also useful in immunotherapy as a cancer treatment.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 4. Document ID: US 20040151761 A1

L9: Entry 4 of 32 File: PGPB Aug 5, 2004

DOCUMENT-IDENTIFIER: US 20040151761 A1
TITLE: Methods and compositions utilizing astaxanthin

Detail Description Paragraph:
[0033] Astaxanthin (3,3'-dihydroxy-beta, beta-carotene-4,4'-dione), an oxycarotenoid or alhpa-hydroxy-ketocarotenoid, is a potent antioxidant (Martin et al., 1999). It is commonly used in aquaculture and in the poultry industry as a feed additive, primarily due to its red pigment. The antioxidant activity of astaxanthin against certain reactive oxygen species has been observed to be higher than that of beta-carotene, canthaxanthin, lutein, alpha-tocopherol, tunaxanthin, and zeaxanthin (Naguib, 2000; Miki, 1991). Rainbow trout fed astaxanthin-rich yeast have demonstrated increased ability to reduce oxidized oil-induced oxidative stress (Nakano et al., 1999) and lower levels of serum lipid peroxides and transaminase activities (Nakano et al., 1995 and Nakano et al., 1996). Whether directly or indirectly related to its antioxidant activity, astaxanthin enhanced both humoral (Jyonouchi et al., 1995) and cell-mediated (Chew et al., 1999b) immune responses, and inhibited mammary (Chew et al., 1999a) and bladder (Tanaka et al., 1994) tumor growth in rodents. It has also been shown to enhance mitogen-induced splenocyte proliferation (Chew et al., 1999) in mice. In Helicobacter pylori-infected mice fed astaxanthin-rich algae extract, the bacterial load and gastric inflammation were reduced, apparently due to a shift in T-lymphocyte from a Th1 response dominated by IFN-gamma to a mixed Th1/Th2 response with IFN-gamma and IL-4 (Bennedsen et al., 1999).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 5. Document ID: US 20040138415 A1

L9: Entry 5 of 32 File: PGPB Jul 15, 2004

DOCUMENT-IDENTIFIER: US 20040138415 A1
TITLE: Helicobacter proteins, nucleic acids and uses thereof

Detail Description Paragraph:
[0263] For analysis of cytokine release of T cells in response to Helicobacter

polypeptides, responder cells are mixed with polypeptides. Supernatants are collected and added to an enzyme-linked immunosorbent assay (ELISA) coated with antibody to the cytokine (e.g. anti-IFN-.gamma. or anti-IL-2 antibody). After washing, rabbit anti-cytokine polyclonal antibody (e.g. anti-IFN-.gamma. or anti-IL-2) is added. Labeled goat anti-rabbit IgG polyclonal is added. Substrate is added and the amount of cytokine released into the supernatant is determined based upon the amount of color developed in the ELISA test.

☐ 6. Document ID: US 20040057926 A1

L9: Entry 6 of 32 File: PGPB Mar 25, 2004

DOCUMENT-IDENTIFIER: US 20040057926 A1
TITLE: Modulation of the immune response through the manipulation of arginine levels

Detail Description Paragraph:
[0283] H. pylori infection induces an inflammatory response characterized by infiltrating polymorphonuclear leukocytes, macrophages and lymphocytes, and the production of several inflammatory cytokines including TNF-60 , IFN-.gamma. and IL8. Tanahashi, T., et al. Infect. Immun. 68, pp. 664-671 (2000); Bauditz, J., et al. Clin. Exp. Immunol. 117, pp. 316-323 (1999); Beales, I. L., et al. Cytokine 9, pp. 514-520 (1997); Sharma, S. A., et al. J. Immunol. 160, pp. 2401-2407 (1998); Yamada, H., et al. Biochem. Pharmacol. 61, pp. 1595-1604 (2001). However, this strong immune response appears to confer little or no protection against H. pylori infection. In vitro models show that virulent strains of H. pylori (carrying the PAI) can impair phagocytosis by delaying actin rearrangement. Allen, L. A., et al. J. Exp. Med. 191, pp. 115-128 (2000). Once phagocytosed, these strains of H. pylori cause the fusion of phagosomes into megasomes, decreasing the killing ability of macrophages. Allen, L. A., et al. J. Exp. Med. 191, pp. 115-128 (2000). In doing so, H. pylori not only delays its own phagocytosis, but also that of other particles particles and bacteria. Ramarao, N., et al. Infect. Immun. 69, pp. 2604-2611 (2001). However, little is known on how H. pylori affects T-cells.

☐ 7. Document ID: US 20040043379 A1

L9: Entry 7 of 32 File: PGPB Mar 4, 2004

DOCUMENT-IDENTIFIER: US 20040043379 A1
TITLE: Method of detectingnucleic acid relating to disease

Detail Description Paragraph:
[0133] For example, it is possible to predict the efficacy of IFN treatment against diseases other than hepatitis C, known to be treated with IFN effectively, such as for example including but not limited to, infectious diseases caused by infection

with virus such as hepatitis C (A, B, C, D, E, F, G types) virus, HIV, influenza virus, herpes virus, adenovirus, human polyomavirus, human papilloma virus, human parbovirus, Mumps virus, human rotavirus, enterovirus, Japanese B Encephalitis virus, dengue virus, rubella virus, and HTLV; and infectious diseases caused by infection with bacteria such as Staphylococcus aureus, hemolytic streptococcus, pathogenic Escherichia coli, enteritis vibrio, Helicobacter pylori, Campylobacter, Vibrio cholerae, dysentery bacilli, salmonellae, Yelsinia, Neisseria gonorrhoeae, Listeria, Leptospira, Legionella, spirochete, Mycoplasma pneumoniae, rickettsiae, chlamydiae, malaria plasmodia, dysentery amoeba and pathogenic fungi; and diseases caused by parasites and Eumycetes.

☐ 8. Document ID: US 20040038329 A1

L9: Entry 8 of 32 File: PGPB Feb 26, 2004

DOCUMENT-IDENTIFIER: US 20040038329 A1
TITLE: Methods for monitoring treatment of helicobacter infection and for predicting the likelihood of successful eradication

Abstract Paragraph:
The present invention relates to methods for monitoring treatment of Helicobacter infection and in particular to methods for monitoring eradication of Helicobacter pylori infection using immunoglobulin G2 (IgG2). The invention also relates to methods for predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection and in particular, to methods for predicting the likelihood of successful eradication including determining the levels of interleukin, interferon-.gamma. and IgG in the subject to be, or being treated.

Summary of Invention Paragraph:
[0001] The present invention relates to methods for monitoring treatment of Helicobacter infection and in particular to methods for monitoring eradication of Helicobacter pylori infection using immunoglobulin G2 (IgG2). The invention also relates to methods for predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection infection and in particular, to methods for predicting the likelihood of successful eradication including determining the levels of interleukin-4, interferon-.gamma. and IgG in the subject to be, or being treated.

Brief Description of Drawings Paragraph:
[0058] FIG. 6 INF-.gamma. production in response to H. pylori acid-glycine extract stimulation in whole blood. Peripheral blood was collected from individual subject and cultured in the presence of graded concentration of H. pylori AGE antigen for 24 hours. Culture supernatants were collected and assayed for IFN-.gamma. by ELISA. Results shown were mean.+-.standard error of the mean. NS: Not Significant.

Detail Description Paragraph:
[0085] Cytokine levels in whole blood culture were measured following the method described previously (Ren et al, Helicobacter 2000; 5:135-41). Briefly, 150 AL of heparinized whole blood was added in triplicate to wells of a 96-well microtitre flat-bottomed plate pre-coated with mouse polyclonal anti-human IL-4 antibody (Endogen, MA, USA). An equal volume of AIM-V medium containing H. pylori AGE at either 0, 1 or 10 .mu.g/mL was also loaded to wells. The cultures were incubated at

37.degree. C. with 5% CO.sub.2 for 24 hours, after which time supernatants were collected for interferon-.gamma. (IFN-.gamma.) assay. The amount of `captured` IL-4 was measured by ELISA as following. Briefly, after washing the plates, biotinylated mouse monoclonal anti-human IL-4 antibody (Endogen, MA, USA) was added (0.5 .mu.g/mL) to wells and incubated for 90 minutes at room temperature. The plates were then washed and incubated for a further 30 minutes at room temperature with streptavidin-conjugated horseradish peroxidase (Selinus, Australia) at a 1:400 dilution. The plates were thoroughly washed with washing buffer and finally incubated for 10 minutes at room temperature with 3,3'-5,5' tetramethyl benzidine (TMB, Sigma-Aldrich, USA) substrate. The reaction was stopped using 1 mol/L H.sub.2SO.sub.4 and optical density at 450 nm (OD 450 nm) was measured in an ELISA plate reader (Bio-Rad 450, Japan). Standard IL-4 (Endogen, MA, USA) was applied for each plate to control plate to plate variation. The limits of sensitivity for IL-4 was 9.4 .mu.g/mL. The amount of IL-4 in samples was determined using a Softmax program (Version 2.3 FPU, USA).

Detail Description Paragraph:
[0097] Significantly lower levels of IL-4 were detected in whole blood stimulated or unstimulated with H. pylori AGE from subjects with eradication failure compared with subjects in whom H. pylori was successfully eradicated ($p<0.05$, 0 and 1.0 .mu.g/mL H. pylori AGE; $p<0.01$, 10 .mu.g/mL H. pylori AGE) or in subjects with untreated infection ($p<0.05$, 10 .mu.g/mL H. pylori AGE) (FIG. 2). IL-4 levels were similar in non-infected and infected subjects, and were not significantly different when compared to subjects with successful eradication (though there was a trend towards increased levels following eradication). Although there was no statistically significant difference in the levels of IFN-.gamma. between the different groups, lower levels were detected in subjects with successful H. pylori eradication (FIG. 3). Low levels of IL-4 secretion were seen in most subjects with ongoing infection with resistant H. pylori, irrespective of the number of courses of of therapy (Table 2).

CLAIMS:

15. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination of interferon-.gamma. (INF-.gamma.) level in a sample from the subject; (ii) comparison of the INF-.gamma. level with a predetermined control or standard INF-.gamma. level, (iii) wherein a level of INF-.gamma. in the sample from the subject below the control or standard INF-.gamma. level is predictive of the likelihood of successful eradication and a level of INF-.gamma. above the control or standard level is predictive of the likelihood of eradication failure.

19. A method according to any one of claims 15 to 18, wherein the control or standard level of IFN-.gamma. is established from analysis of samples obtained from subjects not infected by H. pylorii and/or subjects having successfully eradicated H. pylori and/or subjects infected by H. pylori.

24. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination a combination of IL-4 and/or INF-.gamma. and/or IgG levels in a sample from the subject; (ii) comparison of the IL-4 and/or INF-.gamma. and/or IgG levels with a predetermined control or standard L-4 and/or IF-.gamma. and/or IgG level respectively, wherein a level of IL-4 in the sample from the subject above the control or standard level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard level is predictive of the likelihood of eradication failure, and wherein a level of INF-.gamma. in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IFN-.gamma. above the control or standard level is predictive of the likelihood of eradication failure, and wherein a level of IgG in the sample from the subject below the control or standard

level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

☐ 9. Document ID: US 20040019017 A1

L9: Entry 9 of 32 File: PGPB Jan 29, 2004

DOCUMENT-IDENTIFIER: US 20040019017 A1
TITLE: Caspase inhibitor prodrugs

Detail Description Paragraph:
[0092] The above-described compounds and compositions are particularly useful in therapeutic applications relating to an IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, a proliferative disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, retinal disorders, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, asthma, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyrbiditis, Grave's disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis, scarring, graft vs host disease, organ transplant rejection, organ apoptosis after burn injury, osteoporosis, leukemias and related disorders, myelodysplastic syndrome, multiple myeloma-related bone disorder, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, multiple myeloma, hemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia, epilepsy, myocardial ischemia, acute and chronic heart disease, myocardial infarction, congestive heart failure, atherosclerosis, coronary artery bypass graft, spinal muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, ulcerative colitis, traumatic brain injury, spinal cord injury, hepatitis-B, hepatitis-C, hepatitis-G, yellow fever, dengue fever, Japanese encephalitis, various forms of liver disease, renal disease, polycystic kidney disease, H. pylori-associated gastric and duodenal ulcer disease, HIV infection, tuberculosis, and meningitis. The compounds and compositions are also useful in treating complications associated with coronary artery bypass grafts. The compounds and compositions are also useful for decreasing IGIF or IFN-.gamma. production. The compounds and compositions are also useful in immunotherapy for treatment of cancer.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 10. Document ID: US 20030180260 A1

L9: Entry 10 of 32 File: PGPB Sep 25, 2003

DOCUMENT-IDENTIFIER: US 20030180260 A1
TITLE: Immunotherapy or treating bacterial or viral infection at mucosal surfaces with probiotics, and compositions therefor

Detail Description Paragraph:
[0058] Further, the probiotics of the present invention may be used in conjunction with other treatments, to enhance or assist in their efficacy. For example, approximately 20% of patients treated with antibiotics for H. pylori infection fail to eradicate the organism. This resistance to antibiotic therapy may be due to a shift towards a Th0 response (ie, less IFN-.gamma. and more IL-4). Administration of of a probiotic prior to, in conjunction with or subsequent to antibiotic therapy can be beneficial by switching back to a more dominant Th1 response and thus supplementing or assisting the antibiotic therapy to eradicate the organism in such patients.

☐ 11. Document ID: US 20030149027 A1

L9: Entry 11 of 32 File: PGPB Aug 7, 2003

DOCUMENT-IDENTIFIER: US 20030149027 A1
TITLE: 1,5-benzodiazepine compounds, their production and use

Summary of Invention Paragraph:
[0203] The compounds (I) of the invention or the salts thereof have low toxicity, and exhibit little adverse side effects, so that they can be used as prophylactics, diagnostic agents, or remedies for mammals (e.g., human, cattle, horse, dog, cat, monkey, mouse and rat, especially, human). The compounds (I) of the invention or the salts thereof inhibit or regulate production or secretion of a variety of hormones, growth factors and physiologically active substances. The "hormones" include, for example, growth hormone (GH), thyroid stimulating hormone (TSH), prolactin, insulin, glucagon, and the like. The "growth factors" include, for example, IGF-1 and the like. The "physiologically active substances" include, for example, vasoactive intestinal polypeptide (VIP), gastrin, glucagon-like peptide-1, amylin, substance-P, CGRP, CCK (cholecystokinin), amylase, and the like. Also, "physiologically active substances" include interleukins and cytokines such as TNF-.alpha., etc., and the like. Furthermore, these compounds function through various intracellular signal transduction systems with which somatostatin participates. The intracellular signal transduction systems include intracellular signal transduction systems that involves adenylate cyclase, K.sup.+ channels, Ca.sup.2+ channels, protein dephosphorylation, phospholipase C/inositol trisphosphate production systems, MAP kinase, Na.sup.+/H.sup.+ exchanger systems, phospholipase A2, transcription factors such as NF-.gamma.B, etc. The compounds (I) of the invention or the salts thereof regulate a direct or indirect cell proliferation inhibitory action or apotosis in which somatostatins participate. Therefore, the compounds (I) of the invention or the salts thereof are useful in regulating diseases associated with disorders of production or secretion of such hormones, growth factors, physiologically active substances and etc.; diseases associated with disorders of the above intracellular signal transduction systems (e.g., diseases associated with excess enhancement or inhibition, etc.); disorders of regulation of cell proliferation. Concretely, they can be used (1) as agents for treatment of tumors such as acromegaly, TSH-producing tumors, nonsecretory (afunctional) hypophysial

tumors, ectopic ACTH (adrenocorticotrophin)-pro- ducing tumors, medullar thyroid carcinoma, VIP-producing tumors, glucagon-producing tumors, gastrin-producing tumors, insulinoma and carotinoid tumor, etc., (2) as agents for treatment of insulin-dependent or non-insulin dependent diabetes or a variety of diseases associated with them, for example, diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, Down's syndrome and orthostatic hypotension, etc., (3) as agents for improvement of hyperinsulinemia or for treatment of obesity and overeating through inhibition of appetite, etc., (4) as agents for treatment of acute pancreatitis, chronic pancreatitis, pancreal/intestinal fistula, hemorrhagic ulcer, peptic ulcer, gastritis, hyperacidity, through inhibition or regulation of external secretion at digestive tracts, etc., (5) as agents for improvement of various symptoms associated with the Helicobacter pylori infection, for example, inhibitors of gastrin hypersecretion, etc., (6) as agents for inhibition of amylase secretion associated with endoscopic cholangiopancreatography, and agents for prognostic treatment of surgical operation of pancreas, etc., (7) as agents for treatment of diarrhea caused by small intestinal malabsorption, promotion of secretion or dyskinesia of the digestive tracts (for example, short bowel syndrome), diarrhea caused by the drugs for cancer chemotherapy, diarrhea caused by congenital small intestine atrophy, diarrhea caused by neuroendocrine tumors such as VIP-producing tumors, etc., diarrhea caused by AIDS, diarrhea caused by graft versus host reaction associated with bone marrow transplantation, diarrhea caused by diabetes, diarrhea caused by celiac plexus blocking, diarrhea caused by systemic sclerosis and diarrhea caused by eosinophilia, etc., (8) as agents for treatment of dumping syndrome, irritable colitis, Crohn disease and inflammatory bowel disease, etc., (9) as agents for treatment of various cancers having growth-dependency on insulin, IGF-1 or other growth factors, or tumors or cancers due to the disorders of inhibiting cell growth caused by other reasons (e.g., thyroid cancer, large bowel cancer, breast cancer, prostatic cancer, small cell lung cancer, non-small cell cancer, pancreatic cancer, stomach cancer, cholangiocarcinoma, hepatic cancer, vesical cancer, ovarian cancer, uterine cancer, melanoma, osteosarcoma, chondrosarcoma, malignant pheochromocytoma, neuro-blastoma, brain tumors, thymoma, renal cancers), leukemia (e.g., leukemia of basophilic leukocyte, chronic lymphocytic leukemia, chronic myeloid leukemia, Hodgkin disease, and non-Hodgkin lymphoma) (agents for treatment of these cancers can be used solely or in combination with other anticancer agents such as Tamoxifen, LHRH agonists, LHRH antagonists, interferon-.alpha., interferon-.beta., interferon-.gamma., interleukin-2, etc.), (10) as agents for prevention and treatment of hypertrophic cardiomyopathy, arteriosclerosis, valvular disease, myocardiac infarction (especially, myocardiac infarction post percutaneous transluminal coronary arterioplasty) and reangioplasty, etc., (11) as agents for treatment of hemorrhage of esophageal varicosis, cirrhosis and peripheral blood vessel disorders, etc., (12) as agents for treatment of diseases associated with general or local inflammation, for example, polyarteritis, rheumatoid arthritis, psoriasis, sunburn, eczema and allergy (e.g., asthma, atopic dermatitis, allergic rhinitis, etc.) because they inhibit or regulate the secretion of physiologically active substances acting on the immune system (e.g., Substance P, tachykinin, cytokines, etc.), (13) as agents for treatment of dementia (e.g., Alzheimer disease, Alzheimer-type senile dementia, vascular/multi-infarct dementia, etc.), headache, migraine, schizophrenia, epilepsy, depression, generalized anxiety disorder, sleep disorder, and multiple sclerosis, etc., because they influence the production and secretion of nerve regulating factors, (14) as analgesics, (15) as agents for treatment of acute bacterial meningitis, acute virus encephalitis, adult respiratory distress syndrome, bacterial pneumonia, severe systemic mycotic infection, tuberculosis, spinal damage, bone fracture, hepatic failure, pneumonia, alcoholic hepatitis, virus A hepatitis, virus B hepatitis, virus C hepatitis, AIDS infection, human papilloma virus infection, influenza infection, metastasis of cancer, multiple myeloma, osteomalacia, osteoporosis, bone Paget disease, oesophagitis, nephritis, renal failure, sepsis, septic shock, hypercalcemia, hypercholesterolemia, hyperglyceridemia, hyperlipemia, systemic lupus erythematosus, transient ischemic attach, alcoholic hepatitis, etc., (16) for cure of organ transplantation, burns,

trauma, alopecia, etc., (17) for oculopathy (e.g., glaucoma, etc.), (18) for imaging imaging of tumors having somatostatin receptors after incorporating a radioactive substance (e.g., .sup.125I, .sup.111In, etc.) to the present compound either directly or through a suitable spacer, and (19) for targeting of tumors having a somatostatin receptor by incorporating an anti-cancer drug to the present compound directly or through a suitable spacer.

☐ 12. Document ID: US 20030039653 A1

L9: Entry 12 of 32 File: PGPB Feb 27, 2003

DOCUMENT-IDENTIFIER: US 20030039653 A1
TITLE: Methods of enhancing T cell responsiveness

Detail Description Paragraph:
[0051] Peptide epitopes can also be derived from any of a variety of infectious microorganisms. It is understood that relevant cells need not be classical APC but can be any B7-H 1-expressing cells (or cells in which B7-H1 expression is inducible by, for example, IFN-.gamma.) infected with an appropriate infectious microorganism. microorganism. Such cells include, without limitation, T cells, tissue epithelial cells, endothelial cells, and fibroblasts. Thus, the methods of the invention can be applied to the treatment of infections by any of a wide variety of infectious microorganisms. While such microorganisms will generally be those that replicate inside a cell (commonly designated intracellular pathogens), since APC presenting peptide epitopes derived from infectious microorganism that are not necessarily in the relevant APC, the methods of the invention can also be applied to situations involving infectious microorganisms that replicate extracellularly or in cells that do not express B7-H1. Relevant microorganisms can be viruses, bacteria, mycoplasma, fungi (including yeasts), and protozoan parasites and specific examples of such microorganisms include, without limitation, Mycobacteria tuberculosis, Salmonella enteriditis, Listeria monocytogenes, M. leprae, Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Borrelia burgdorferi, Actinobacillus pleuropneumoniae, Helicobacter pylori, Neisseria meningitidis, Yersinia enterocolitica, Bordetella pertussis, Porphyromonas gingivalis, mycoplasma, Histoplasma capsulatum, Cryptococcus neoformans, Chlamydia trachomatis, Candida albicans, Plasmodium falciparum, Entamoeba histolytica, Toxoplasma brucei, Toxoplasma gondii, Leishmania major human immunodeficiency virus 1 and 2, influenza virus, measles virus, rabies virus, hepatitis virus A, B, and C, rotaviruses, papilloma virus, respiratory syncytial virus, feline immunodeficiency virus, feline leukemia virus, and simian immunodeficiency virus.

☐ 13. Document ID: US 20020107368 A1

L9: Entry 13 of 32 File: PGPB Aug 8, 2002

DOCUMENT-IDENTIFIER: US 20020107368 A1

TITLE: Helicobacter proteins, gene sequences and uses thereof

Detail Description Paragraph:
[0253] For analysis of cytokine release of T cells in response to Helicobacter polypeptides, responder cells are mixed with polypeptides. Supernatants are collected and added to an enzyme-linked immunosorbent assay (ELISA) coated with antibody to the cytokine (e.g. anti-IFN-.gamma. or anti-IL-2 antibody). After washing, rabbit anti-cytokine polyclonal antibody (e.g. anti-IFN-.gamma. or anti-IL-2) is added. Labeled goat anti-rabbit IgG polyclonal is added. Substrate is added and the amount of cytokine released into the supernatant is determined based upon the amount of color developed in the ELISA test.

☐ 14. Document ID: US 20010044416 A1

L9: Entry 14 of 32 File: PGPB Nov 22, 2001

DOCUMENT-IDENTIFIER: US 20010044416 A1
TITLE: Immunostimulatory nucleic acids for inducing a Th2 immune response

Summary of Invention Paragraph:
[0003] The existence of functionally polarized T cell responses based on the profile of cytokines secreted by CD4+ T helper (Th) cells has been well established. In general, Th1 cells secrete interferon-gamma (IFN-.gamma.), interleukin (IL)-2, and tumor necrosis factor-beta (TNF.beta.), and are important in macrophage activation, the generation of both humoral and cell-mediated immune responses and phagocyte-dependent protective responses. Th2 cells secrete IL-4, IL-5, IL-10, and IL-13 and are more important in the generation of humoral immunity, eosinophil activation, regulation of cell-mediated immune responses, control of macrophage function and the stimulation of particular Ig isotypes (Morel et al., 1998, Romagnani, 1999). Th1 cells generally develop following infections by intracellular pathogens, whereas Th2 cells predominate in response to intestinal nematodes. In addition to their roles in protective immunity, Th1 and Th2 cells are responsible for different types of immunopathological disorders. For example, Th1 cells predominate in organ specific autoimmune disorders, Crohn's disease, Helicobacter pylori-induced peptic ulcer, acute solid organ allograft rejection, and and unexplained recurrent abortion, whereas Th2 cells predominate in Omenn's syndrome, systemic lupus erythematosus, transplantation tolerance, chronic graft versus host disease, idiopathic pulmonary fibrosis, and progressive systemic sclerosis, and are involved in triggering of allergic reactions (Romagnani 1999, Singh et al., 1999). Therefore, for both prophylactic and therapeutic purposes, depending on the particular disease, a preference for either Th1 or Th2 type responses exists.

Detail Description Table CWU:
1 Crohn's disease/IBD Kakazu T et al., Type I T-helper cell predominance in granulomas of Crohnrs disease. Am J Gastroenterol 1999 Aug;94(8):2149-55; Monteleone G et al., Bioactive IL-18 expression is up-regulated in Crolin's disease. J Immunol 1999 Jul 1;163(1):143-7; Camoglio L et al., Altered expression of interferon-gamma and interleukin- 4 in inflammatory bowel disease. Inflamm Bowel Dis 1998 Nov;4(4):285-90; Plevy SE et al., A role for TNF-alpha and mucosal T helper-1 cytokines in the pathogenesis of Crohnts disease. J Immunol 1997 Dec 15;159(12):6276-82; Noguchi M et al., Enhanced interferon-gamma production and B7-2

expression in isolated intestinal mononuclear cells from patients with Crohnts disease. J Gastroenterol 1995 Nov;30 Suppl 8:52-5. H. pylori Hida N et al., Increased expression of IL-10 and IL-12 (p40) mRNA in Helicobacter pylori infected gastric mucosa: relation to bacterial cag status and peptic ulceration. J Clin Pathol 1999 Sep;52(9):658-64; Mattapallil JJ et al., A predominant Th1 type of immune response is induced early during acute Helicobacter pylori infection in rhesus macaques. Gastroenterology 2000 Feb;118(2):307-15. Autoimmune Okazaki K et al., Autoimmune-related pancreatitis is associated with pancreatitis autoantibodies and a Th1/Th2-type cellular immune response. Gastroenterology 2000 Mar;118(3):573-81. Chronic hepatitis C Bertoletti A et al., Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. Gastroenterology 1997 Jan;112(1):193-9; Quiroga JA et al., Induction of interleukin- 12 production in chronic hepatitis C virus infection correlates with the hepatocellular damage. J Infect Dis 1998 Jul;178(1):247-51. Behcet's Syndrome Sugi-Ikai N et al., Increased frequencies of interleukin-2- and interferon- gamma-producing T cells in patients with active Behcet's disease. Invest Ophthalmol Vis Sci 1998 May;39(6):996-1004. PBC Dienes HP et al., Bile duct epithelia as target cells in primary biliary cirrhosis and primary sclerosing cholangitis. Virchows Arch 1997 Aug;43 1(2): 119-24; Tjandra K et al., Progressive development of a Thi-type hepatic cytokine profile in rats with experimental cholangitis. Hepatology 2000 Feb;3 1(2):280-90; Harada K et al., In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: predominance of the Th1 subset. Hepatology 1997 Apr;25(4):79 1-6. PSC Dienes HP et al., Bile duct epithelia as target cells in primary biliary cirrhosis and primary sclerosing cholangitis. Virchows Arch 1997 Aug;43 1(2): 119-24; Tjandra K et al., Progressive development of a Th1-type hepatic cytokine profile in rats with experimental cholangitis. Hepatology 2000 Feb;31(2):280-90. Sarcoidosis Moller DR, Cells and cytokines involved in the pathogenesis of sarcoidosis. Sarcoidosis Vasc Difuse Lung Dis 1999 Mar;16(1):24-31; Moller DR et al., Enhanced expression of IL-12 associated with Th1 cytokine profiles in active pulmonary sarcoidosis. J Immunol 1996 Jun 15; 156(12):4952-60. Atherosclerosis Frostegard J et al., Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th 1) and macrophage-stimulating cytokines. Atherosclerosis 1999 Jul; 145(1): 33-43. Acute GvHD Ochs LA et al., Cytokine expression in human cutaneous chronic graft- versus-host disease. Bone Marrow Transplant 1996 Jun;17(6):1085-92; Williamson B et al., Neutralizing IL-12 during induction of murine acute graft-versus-host disease polarizes the cytokine profile toward a Th2- type alloimmune response and confers long term protection from disease. J Immunol 1997 Aug 1;159(3): 1208-15. Glomerulonephritis Kitching AR et al., IFN-gamma mediates crescent formation and cell- mediated immune injury in murine glomerulonephritis. J Am Soc Nephrol 1999 Apr;10(4):752-9; Holdsworth SR et al., Th1 and Th2 T helper cell subsets affect patterns of injury and outcomes in glomerulonephritis. Kidney Int 1999 Apr;55(4):1198-216. Wegener's Gross WL et al., Pathogenesis of Wegener's granulomatosis. Ann Med granulomatosis Interne (Paris) 1998 Sep; 149(5):280-6. Anti-GBM disease Kalluri R et al., Susceptibility to anti-glomerular basement membrane disease and Goodpasture syndrome is linked to MHC class II genes and the emergence of T cell-mediated immunity in mice. J Clin Invest 1997 Nov 1;100(9):2263-75; Coelho SN et al., Immunologic determinants of susceptibility to experimental glomerulonepliritis: role of cellular immunity. Kidney Int 1997 Mar;51(3):646-52. Lepidi H et al., Local expression of cytokines in idiopathic inflammatory myopathies. Neuropathol Appl Neurobiol 1998 Feb;24(1): 73-9. Siogren's syndrome Kolkowski BC et al., Th1 predominance and perform expression in minor salivary glands from patients with primary Sjogren's syndrome. J Autoimmun 1999 Aug;13(1):155-62. Lyme arthritis Yin Z et al., T cell cytokine pattern in the joints of patients with Lyme arthritis and its regulation by cytokines and anticytokines. Arthritis Rheum 1997 Jan;40(1):69-79. Rheumatoid arthritis Kusaba M et al., Analysis of type 1 and type 2 T cells in synovial fluid and peripheral blood of patients with rheumatoid arthritis. J Rheumatol 1998 Aug;25(8):1466-71.

☐ 15. Document ID: US 6800744 B1

L9: Entry 15 of 32 File: USPT Oct 5, 2004

DOCUMENT-IDENTIFIER: US 6800744 B1
TITLE: Nucleic acid and amino acid sequences relating to Streptococcus pneumoniae for diagnostics and therapeutics

Detailed Description Paragraph Table (4):
10657137_c1_67 79 2682 552 183 153 1.20E-10 [ac:p20665] [gn:piv] [or:moraxella bovis] [de:pilin gene inverting protein (pivml)] [sp:p20665] [db:swissprot] 10664092_f2_21 80 2683 333 110 154 8.70E-111 [ac:p39140] [gn:deor] [or:bacillus subtilis] [de:deoxyribonucleoside regulator] [sp:p39140] [db:swissprot] 10683128_f3_35 81 2684 459 152 119 2.70E-06 [ln:tnaf00605] [ac:af000605] [pn:insect intestinal mucin iim14] [or:trichoplusia ni] [sr:cabbage looper] [db:genpept-inv] [de:trichoplusia ni insect intestinal mucin iim14 mrna, complcte cds.] [le:38] [re:2404] [di:direct] 1070277_f2_33 82 2685 555 184 479 1.00E-45 [ac:s62019] [pn:hypothetical protein ydr540c:hypothetical protein d3703.8] [or:saccharomyces cerevisiae] [db:pir] [mp:4r] 1072138_c3_65 83 2686 1293 430 881 2.60E-88 [ac:h69627] [pn:signal recognition particle ftsy] [gn:ftsy] [or:bacillus subtilis] [db:pir] 1072885_c1_9 84 2687 381 126 210 3.30E-17 [ac:p46319] [gn:celc] [or:bacillus subtilis] [ec:2.7.1.69] [de:(ec 2.7.1.69) (eiii-cel)] [sp:p46319] [db:swissprot] 10737661_f3_14 85 2688 585 194 264 6.20E-23 [ac:p42313] [gn:yxjb:n15i] [or:bacillus subtilis] [de:hypothetical 31.5 kd protein in katb 3'region] [sp:p42313] [db:swissprot] 10737782_f1_47 86 2689 183 60 74 0.041 [ln:celf38b6] [ac:u40060] [gn:f38b6.3] [or:caenorhabditis elegans] [sr:caenorhabditis elegans strain=bristol n2] [db:genpept-inv] [de:caenorhabditis elegans cosmid f38b6.] [le:25381:26844:27050:27814] [re:25476:26998:27138:27926] [di:directjoin] 10738758_f3_26 87 2690 198 65 50 0.72 [ln:cef49e11] [ac:z70308] [pn:f49e11.1] [or:caenorhabditis elegans] [db:genpept-inv] [de:caenorhabditis elegans cosmid f49e11, complete sequence.] [nt:similarity to s.pombe serine/threonine protein] [le:3290:3550:4496:4868] [re:3338:3724:4811:5131) [di:di 10740875_c2_18 88 2691 375 124 215 9.60E-18 [ac:p25614] [gn:ycr13c] [or:saccharomyces cerevisiae] [sr:,baker's yeast] [de:very hypothetical 22.8 kd protein in pgk1 region] [sp:p25614] [db:swissprot] 10740937_f1_1 89 2692 330 109 69 0.028 [ac:p37188:p76412] [gn:gatb] [or:escherichia coli] [ec:2.7.1.69] [de:(ec 2.7.1.69)] [sp:p37188:p764121] [db:swissprot] 10740937_f2_2 90 2693 327 108 72 0.014 [ac:p37188:p76412] [gn:gatb] [or:escherichia coli] [ec:2.7.1.69] [de:(ec 2.7.1.69)] [sp:p37188:p76412] [db:swissprot] 10741542_c3_109 91 2694 252 83 63 0.3 [ac:f64569] [pn:hypothetical protein hp0398] [or:helicobacter pylori] [db:pir] 10744551_f3_7 92 2695 231 76 182 3.00E-14 [ac:d69868] [pn:conserved hypothetical protein ykvm] [gn:ykvm] [or:bacillus subtilis] [db:pir] 10745937_f3_29 93 2696 621 206 233 1.20E-19 [ac:p96707] [gn:ydgi] [or:bacillus subtilis] [ec:1.-.-.-] [de:putative nad(p)h nitroreductase,] [sp:p96707] [db:swissprot] 10761062_c1_10 94 2697 1620 539 2010 5.90E-208 [ac:p13242] [gn:ctra] [or:bacillus subtilis] [ec:6.3.4.2] [de:ctp synthase, (utp--ammonia ligase) (ctp synthetase)] [sp:p13242] [db:swissprot] 10804062_f2_6 95 2698 1107 368 766 3.90E-76 [ac:p77212] [gn:ykgc] [or:escherichia coli] [de:intergenic region] [sp:p77212] [db:swissprot] 10817943_f2_4 96 2699 864 287 661 5.30E-65 [ac:f70016] [pn:purine permease homolog yunk) [gn:yunk] [or:bacillus subtilis] [db:pir] 10937882_f2_36 97 2700 2334 777 2200 4.30E-228 [ac:p50849] [gn:pnpa:comr] [or:bacillus subtilis] [ec:2.7.7.8] [de:phosphorylase) (pnpase)] [sp:p50849] [db:swissprot] 10939413_c3_87 98 2701 1074 357 396 6.30E-37 [ac:a64433] [pn:spore coat polysaccharide biosynthesis protein c homolog] [cl:erythromycin resistance protein] [or:methanococcus jannaschii]

[db:pir] [mp:rev1008090-1006930] 10941387_c3_86 99 2702 510 169 94 0.013 [ac:h64431] [ac:h64431] [pn:glycosyl transferase,] [or:methanococcus jannaschii] [ec:2.4.-.-] [db:pir] [mp:for996513-997385) 10944035_f3_29 100 2703 399 132 246 5.00E-21 [ln:soorfs] [ac:z79691] [pn:orfb] [gn:yorfb] [or:streptococcus pneumoniae] [db:genpept- bct] [de:s.pneumoniae yorf[a,b,c,d,e], fts1, pbpx and regr genes.] [le:1914] [re:2372] [di:complement] 10956442_c2_90 101 2704 519 172 139 3.80E-09 [ln:llpflmg13] [ac:aj000325] [pn:putative membrane protein] [gn:orfa] [or:lactococcus lactis] [db:genpept-bct] [de:lactococcus lactis pfl gene (strain mg1363).] [le:270] [re:1187] [di:direct] 10964383_c1_11 102 2705 819 272 89 0.36 [ac:p37963] [gn:spovid] [or:bacillus subtilis] [de:stage vi sporulation protein d] [sp:p37963] [db:swissprot] 10969461_f3_2 103 2706 474 157 301 7.40E-27 [ac:b53293] [pn:flm3 region hypothetical protein 2] [or:synechococcus sp.] [db:pir] 10970338_c1_75 104 2707 294 97 76 0.031 [ln:d87074] [ac:d87074] [gn:kiaa0237] [or:homo sapiens] [sr:homo sapiens male bone marrow myeloblast cell line:kg-1 cdna t] [db:genpept-pri2] [de:human mrna for kiaa0237 gene, complete cds.] [nt:similar to to a e.elegans protein encoded in cosmid] [le:476] [10970968_c1_22 105 2708 1284 427 155 4.40E-08 [ln:af017983] [ac:af017983] [pn:gamma-glutamylcysteine synthetase] [gn:gsh1] [or:lycopersicon esculentum] [sr:tomato] [db:genpept-pln] [de:lycopersicon [de:lycopersicon esculentum gamma-glutamylcysteine synthetase (gsh1)mrna, complete cds.] [le:239] [re:1810] [di:direct] 10975167_c1_24 106 2709 717 238 677 1.10E-66 [ac:p30294] [gn:livf:livg] [or:salmonella typhimurium] [de:livf(liv-i protein f)] [sp:p30294] [db:swissprot] 10975292_f2_10 107 2710 750 249 299 1.20E-26 [ln:syngip3124] [ac:m77279] [pn:alpha-amylase] [or:unidentified cloning vector] [sr:cloning vector (sub_species cloning vector pgip3124) dna] [db:genpept-syn] [de:cloning vector pgip3124 with inserted enterococcus faecalisalpha-amylase fusion protein gene 10976587_c1_52 108 2711 375 124 87 0.00052 [ac:p37471] [gn:divic:diva] [or:bacillus subtilis] [de:cell division protein divic] [sp:p37471] [db:swissprot] 10977307_f2_34 109 2712 510 169 116 1.60E-05 [ac:p22560] [or:mus musculus] [sr:,mouse] [de:ifn-response binding factor 1 (irebf-1)] [sp:p22560] [db:swissprot] 10979678_f2_12 110 2713 357 118 268 2.30E-23 [ac:f70009] [pn:conserved hypothetical protein yufq] [gn:yufq] [or:bacillus subtilis] [db:pir] 10985215_f3_4 111 2714 1044 347 60 0.87 [ln:pbu42580] [ac:u42580:u17055:u32570] [gn:a4201] [or:paramecium bursaria chlorella virus 1] [db:genpept-vrl] [de:paramecium bursaria chlorella virus 1, complete genome.] [le:204610] [re:204822] [di:complement] 10991461_c3_85 112 2715 1131 376 853 2.40E-85 [ln:lpalrgene] [ac:y08941] [pn:alanine racemase] [gn:alr] [or:actobacillus plantarum] [db:genpept-bct] [ec:5.1.1.1] [de:l.plantarum alr gene.] [le:226] [re:1353] [di:direct] 10992127_c1_37 113 2716 375 124 63 [ac:s75993] [pn:hypothetical protein] [or:synechocystis sp.] [sr:pcc 6803,, pcc 6803] [sr:pcc 6803,] [db:pir] 10995340_f3_3 114 2717 243 80 171 5.60E-12 [ac:p22976] [gn:recp] [or:streptococcus pneumoniae] [ec:2.2.1.1] [de:probable transketolase, (tk)] [sp:p22976] [db:swissprot] 11016376_f1_3 115 2718 996 331 586 4.70E-57 [ac:b70032] [pn:conserved hypothetical protein yvcl] [gn:yvcl] [or:bacillus subtilis] [db:pir] 11016576_f2_8 116 2719 1620 539 139 1.10E-08 [ln:spnana] [ac:x72967] [or:streptococcus pneumoniae] [db:genpept-bct] [de:s.pneumonia nana gene.] [nt:orf2] [le:193] [re:495] [di:direct] 11019406_f3_7 117 2720 243 80 65 0.076 [ac:p38190] [gn:yb1053w:yb10514] [or:saccharomyces cerevisiae] [sr:,baker's yeast] [de:very hypothetical 13.2 kd protein in ptc3-sas3 intergenic region] [sp:p38190] [db:swissprot] 11021088_f1_12 118 2721 201 66 112 1.80E-08 [ac:c69992] [pn:abc transporter (atp-binding protein) homolog ytgb] [gn:ytgb] [or:bacillus subtilis] [db:pir] 11040942_c1_70 119 2722 237 78 87 0.00035 [ln:ehy14328] [ac:y14328] [pn:3e1 protein] [or:entamoeba histolytica] [db:genpept-inv] [de:entamoeba histolytica mrna for 3e1 protein.] [le:32] [re:418] [di:direct] 11053302_f2_3 120 2723 291 96 56 0.57 [ac:o05239] [gn:yugj] [or:bacillus subtilis]

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw

☐ 16. Document ID: US 6713062 B1

L9: Entry 16 of 32 File: USPT Mar 30, 2004

DOCUMENT-IDENTIFIER: US 6713062 B1
**** See image for Certificate of Correction ****
TITLE: Acinetobacter outer membrane protein and gene sequence compositions and methods

Detailed Description Text (8):
In mouse models and humans, H. pylori is associated with an increase in serum gastrin and gastrin-expressing (G)-cells with a concomitant decrease in somatostatin-expressing D cells. This change appears to follow an increase in interferon-.gamma. expressing Th1 lymphocytes cells. Atrophy of the acid-producing parietal cells leads to metaplastic changes in the stomach. The development of atrophic gastritis leads to decreased colonization by H. pylori and increased colonization by non-H. pylori organisms.

Detailed Description Text (9):
The present invention shows that Acinetobacter causes the same histology as H. pylori on the gastric mucosa. Gastric epithelial cells were isolated by mechanical dissociation. All cell populations were analyzed by flow cytometry. Two months after mice were inoculated with H. pylori or Acinetobacter, the gastric T cell numbers doubled; whereas, an increase in the number of B cells was not observed until 3 months after infection. After 4 months of infection, there was a 3-fold increase in the number of G cells and a doubling in the number of parietal cells. A 3-fold decrease in the number of D-cells occurred in H. pylori and Acinetobacter infected mice. Plasma gastrin and IL-8 levels increased after both H. pylori and Acinetobacter infection. Furthermore, CD8+ cells producing IFN-.gamma. were elevated in the infected mice.

Detailed Description Text (438):
Flow cytometric analysis was used to phenotype (subtype) the T cell response. It was found that there was an increase in the number of CD4+, CD8+ and CD8+ lymphocytes expressing IFN-.gamma. cells in both H. pylori and Acinetobacter inoculated mice compared to uninoculated mice.

Detailed Description Text (454):
In this study, CD8+ cells expressing IFN-.gamma. increased with Acinetobacter infection. Such an observation is typical of a Th1 immune response activated during H. pylori infection (Bamford et al., 1998; Mohammadi et al., 1996; Strober et al., 1997). Furthermore, immunization against IFN-Y resulted in a reduction of gastric inflammation in H. pylori infected mice (Mohammadi et al., 1996). It is also important to note that increased IFN-.gamma. expression during H. pylori infection correlates with an increase in gastrin secretion both in vivo and in vitro (Jassel et al., 1999; Lehmann et al., 1996). The present study also correlates an increase in IFN-.gamma. expression with increased plasma gastrin and G-cell numbers. In addition, a study using isolated canine antral G cells showed stimulation in gastrin release by IFN-.gamma. (Lehmann et al., 1996).

Detailed Description Text (473):
Sambrook, Fritsch, Maniatis, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1989. Saunders et al., "Simple sequence repeats in the Helicobacter pylori genome," Mol. Microbiol., 27:1091-8, 1998. Seery, "Achlorhydria and gastric carcinogenesis," Lancet., 338:1508-9, 1991. Sharma et al., "Intragastric bacterial activity and nitrosation before, during, and after treatment with omeprazole," Br. Med. J. (Clin. Res. Ed)., 289:717-9, 1984.

Singh et al., "Gastrin gene expression is required for the proliferation and tumorigenicity of human colon cancer cells," Cancer Res., 56:4111-4115, 1996. Stockbruegger et al., "Pernicious anaemia, intragastric bacterial overgrowth, and possible consequences," Scand. J. Gastroenterol, 19:355-64, 1984. Strober et al., "Reciprocal IFN-gamma and TGF-beta responses regulate the occurrence of mucosal inflammation," Immunol. Today, 18:61-4, 1997. Sumii et al., "Expression of antral gastrin and somatostatin mRNA in Helicobacter pylori-infected subjects," Am, J. Gastroenterol., 89:1515-1519, 1994. Tang et al., Nature, 356:152-154, 1992. Terres and Pajares, "An increased number of follicles containing activated CD69+ helper T cells and proliferating CD71+B cells are found in H. pylori-infected gastric mucosa," Am. J. Gastroenterol, 93:579-83, 1998. Toh et al., "Mechanisms of disease," New Eng. J. Med., 337:1441-1448, 1997. Tomb, White, Kerlavage et al., "The complete genome sequence of the gastric pathogen Helicobacter pylori," Nature, 388:539-547, 1998. Torres et al., "Stomach as a source of colonization of the respiratory tract during mechanical ventilation: association with ventilator-associated pneumonia." Eur. Respir. J, 9:1729-35, 1996. Ulmer et al., "Heterologous Protection Against Influenza by Injection of DNA Encoding a Viral Protein," Science, 259:1745-1749, 1993. Walsh and Grossman, "Gastrin," N. Engl. J. Med., 292 pt 2:1377-1384, 1975. Wang et al., Proc. Natl. Acad. Sci. USA, 90:4156-4160, 1993. Wang et al., "Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with Helicobacter felis infection," Gastroenterology, 114:675-689, 1998. Wang et al., "Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer," Gastroenterology, 118:36-47, 2000. Weinberger et al., Science, 228:740-742, 1985. Wexler et al., "The isolation and characterisation of a major outer-membrane protein from Bacteroides distasonis," J. Med. Microbiol., 37:165-75, 1992 Whitton et al., J. Virol. 67:(1) 348-352, 1993. Wolf et al., "An Integrated Family of Amino Acid Sequence Analysis Programs," Comput. Appl. Biosci., 4(1):187-191, 1988. Yang et al., "The major outer membrane protein, CD, extracted from Moraxella (Branhamella) catarrhalis is a potential vaccine antigen that induces bactericidal antibodies," FEMS Immunol. Med. Microbiol., 17:187-99, 1997. Zavros et al., "Use of the Mediman Machine to quantify gastric epithelial cells for flow cytometry," Dig. Dis. Sci., In Press, 2000. Zhang et al., Helicobacter pylori infection on the risk of stomach cancer and chronic atrophic gastritis," Cancer Detect. Prev., 23:357-67, 1999.

☐ 17. Document ID: US 6524625 B2

L9: Entry 17 of 32 File: USPT Feb 25, 2003

DOCUMENT-IDENTIFIER: US 6524625 B2
TITLE: Physiologically active extract obtained from indigo plant polygonum tinctorium

Detailed Description Text (5):
The present physiologically active extract containing ethyl acetate-soluble ingredients have at least one or more of the following properties and exert diversified physiological actions on mammals and humans: (1) Inhibiting the growth of gram-positive and gram-negative microorganisms including Helicobacter pylori known as microorganisms which induce gastritis, gastric ulcer, duodenal ulcer, and gastric cancer; (2) Inhibiting the growth of pathogenic virus including influenza virus, vesicular stomatitis virus, herpes simplex virus, vaccinia virus, and cytomegalovirus; (3) Inhibiting the growth of tumor cells of incurable tumors including leukemia-, gastric cancer-, and lung cancer-cells; (4) Entrapping

radicals derived from active oxygen and lipoperoxide that induce malignant tumors, myocardial infarction, cerebral apoplexy, rheumatism, lifestyle related diseases including geriatric diseases, renal disorders, stresses, and aging; (5) Acting on normal and abnormal B-cells, T-cells, nerve cells, epithelial cells of digestive tracts, stem cells of digestive tracts, vascular endothelial cells, skin cells, etc., to regulate the apoptosis of the above cells within normal conditions and to treat/prevent the diseases of digestive organs, circulatory organs, eye, ear, norse, norse, throat, skin, nerve, and bone; (6) Controlling the production of cytokines including interferon-.gamma. and interleukin 10, by immuno-competent cells, which relate to the determination of the balance in vivo between type 1 helper T-cells (Th1) and type 2 helper T-cells (Th2) to control the balance within the normal conditions and to treat/prevent the diseases such as autoimmune diseases, and hepatic disorder-, renal disorder-, pancreatic disorder-, and graft-versus-host reaction-related diseases; and (7) Inhibiting the expression of nitrogen monoxide synthetic enzymes by cells in vivo, induced by cytokines and endotoxins, and inhibiting the formation of nitrogen monoxide to treat/prevent diseases such as autoimmune diseases, allergic diseases, inflammatory diseases, malignant tumors, renal disorders, and lung disorders.

CLAIMS:

4. The extract according to claim 1 wherein the microorganisms are selected from the group consisting of Helicobacter pylon; wherein the pathogenic viruses are selected from the group consisting of influenza virus, vesicular stomatitis virus, herpes simplex virus, vaccinia virus, and cytomegalovirus; wherein the tumor cells of incurable tumors are selected from the group consisting of leukemia, gastric cancer, and lung cancer cells; and wherein the cytokines are selected from the group consisting of interferon-gamma and interleukin 10.

☐ 18. Document ID: US 6482618 B2

L9: Entry 18 of 32 File: USPT Nov 19, 2002

DOCUMENT-IDENTIFIER: US 6482618 B2
TITLE: Self-enhancing, pharmacologically controllable expression systems

Brief Summary Text (106):
h) The Therapy of Chronic Infectious Diseases Target cell: liver cell lymphocyte and/or macrophage epithelial cell endothelial cell Promoters: virus-specific cell-specific virus-specific or cell-specific and cell cycle-specific Structural genes, for example for a protein which exhibits cytostatic or cytotoxic effects. (Examples of cytotoxic or cytostatic proteins have already been cited in the section entitled Tumour therapy.) an enzyme (in this regard, see the section entitled Tumour therapy) which cleaves a precursor of an antiviral or cytotoxic substance, thereby forming the active substance. Structural genes for antiviral proteins cytokines and growth factors possessing antiviral activity. Examples of these are IFN-.alpha. IFN-.beta. IFN-.gamma. TNF.beta. TNF.alpha. IL-1 TGF.beta. antibody having a specificity which inactivates the relevant virus, or its V.sub.H - and VL-containing fragments, or its V.sub.H and V.sub.L fragments which are connected by way of a linker, which fragments can be prepared as already described in Section 2). Examples of antibodies against viral antigens are: anti-HBV anti-HCV anti-HSV

anti-HPV anti-HIV anti-EBV anti-HTLV anti-Coxsackie virus anti-Hantaan virus a rev-binding protein. These proteins bind to the rev RNA and inhibit rev-dependent posttranscriptional steps in retroviral gene expression. Examples of rev-binding proteins are: RBP9-27 RBP1-8U RBP1-8D pseudogenes of RBP1-8 for ribozymes which digest the mRNA of genes for cell cycle control proteins or the mRNA of viruses. Ribozymes which are catalytic for HIV have been reviewed, for example, by Christoffersen et al., J. Med. Chem. 38, 2033 (1995). Structural genes for antibacterial proteins Examples of the antibacterial proteins are antibodies which neutralize bacterial toxins or which opsonize bacteria. Examples of these antibodies antibodies are antibodies against C orB meningococci E. coli Borrelia Pseudomonas Helicobacter pylori Staphylococcus aureus

☐ 19. Document ID: US 6428985 B1

L9: Entry 19 of 32 File: USPT Aug 6, 2002

DOCUMENT-IDENTIFIER: US 6428985 B1
TITLE: Immunosuppressive structural definition of IL-10

Detailed Description Text (213):
The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference without disclaimer. Allen, Staley, Sidman, "Differential cytokine expression in acute and chronic murine graft-versus-host-disease," Eur. J Immunol., 23:333-337, 1993. Allione, Consalvo, Nanni, Lollini, Cavallo, Giovarelli, Forni, Gulino, Colombo, Dellabona, Blankenstein, Rosenthal, Gansbacher, Bosco, Musso, Gusella, Forni, "Immunizing and curative potential of replicating and nonreplicating murine mammary adenocarcinoma cells engineered with interleukin (IL)-2, IL-4, IL-6, IL-7, IL-10, tumor necrosis factor .alpha., granulocyte-macrophage colony-stimulating factor, and .gamma.-interferon gene or admixed with conventional adjuvants," Cancer Research, 54:6022-6026, 1994. Asadullah, Sterry, Stephanek, Jasulaitis, Leupold, Audring, Volk, Docke, "IL-10 is a key cytokine in psoriasis," J. Clin. Invest., 101:783-794, 1998. Baan, Van Emmerik, Balk, Quint, Mochtar, Jutte, Niesters, Weimar, "Cytokine mRNA expression in endomycardial biopsies during acute rejection from human heart transplants," Clin. Exp. Immunol., 97:293-298, 1994. Bacchetta, Bigler, Touraine, Parkman, Tovo, Abrams, de Waal Malefyt, de Vries, Roncarolo, "High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells," J. Exp. Med., 179:493-502, 1994. Beatty, Krams, Martinez, "Involvement of IL-10 in the autonomous growth of EVB-transformed B cell lines," J. Immunol., 158:4045-4051, 1997. Beissert, Hosoi, Grabbe, Asahina, Granstein, "IL-10 inhibits tumor antigen presentation by epidermal antigen-presenting cells," J. Immunol., 154:1280-1286, 1995. Berg, Leach, Kuhn, Rajewsky, Muller, Davidson, Rennick, "Interleukin 10 but not interleukin 4 is a natural suppressant of cutaneous inflammatory responses," J. Exp. Med., 182:99-108, 1995. Berkman, John, Roesems, Jose, Barnes, Chang, "Inhibition of macrophage inflammatory protein-1 .alpha. expression by IL-10," J. Immunol., 155:4412-4418, 1995. Bishop, Rokahr, Napoli, McCaughan, "Intragraft cytokine mRNA levels in human liver allograft rejection analysed by reverse transcription and semiquantitative polymerase chain reaction amplification," Transplant Immunol., 1:253-261, 1993. Bogdan, Vodovotz, Nathan, "Macrophage deactivation by interleukin 10," J. Exp. Med., 174:1549-1555, 1991. Bovolenta, Gasperini, McDonald, Cassatella, "High affinity receptor for IgG (Fc.gamma.RI/CD64) gene and STAT protein binding to the

IFN-.gamma. response region (GRR) are regulated differentially in human neutrophils and monocytes by IL-10," J. Immunol., 160:911-919, 1998. Buer, Lanoue, Franzke, Garcia, von Boehmer, Sarukhan, "Interleukin 10 secretion and impaired effector function of major histocompatibility complex class II-restricted T cells anergized in vivo," J. Exp. Med., 187:177-183, 1998. Burke, Ciancio, Cirocco, Markou, Coker, Roth, Nery, Esquenazi, Miller, "Association of Interleukin-10 with rejection-sparing effect in septic kidney transplant recipients," Transplantation, 61:1114-1116, 1995. Cassatella, Meda, Gasperini, Calzetti, Bonora, "Interleukin 10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation," J. Exp. Med., 179:1695-1699, 1994. Chang, Furue, Tamaki, "Selective regulation of ICAM-1 and major histocompatibility complex class I and II molecule expression on epidermal Langerhans cells by some of the cytokines released by keratinocytes and T cells," Eur. J. Immunol., 24:2889-2895, 1994. Crawley, Williams, Mander, Brennan, Foxwell, "Interleukin-10 stimulation of phosphatidylinositol 3-kinase and p70 S6 kinase is required for the proliferative but not the antiinflammatory effects of the cytokine," J. Biol Chem., 271:16357-16362, 1996. Cunningham, Dunn, Yacoub, Rose, "Local production of cytokines in the human cardiac allograft," Transplantation, 57:1333-1337, 1994. D'Andrea, Aste-Amezaga, Valiante, Ma, Kubin, Trinchiere, "Interleukin 10 (IL-10) inhibits human lymphocyte interferon .gamma.-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells," J. Exp. Med., 178:1041-1048, 1993. Daheshia, Kuklin, Kanangat, Manickan, Rouse, "Suppression of ongoing ocular inflammatory disease by topical administration of plasmid DNA IL-10," J. Immunol., 159:1945-1952, 1997. Danzer, Kirchner, Rink, "Cytokine interactions in human mixed lymphocyte culture," Transplantation, 57:1638-1642, 1994. de Waal Malefyt, Abrams, Bennett, Figdor, de Vries, "Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes," J. Exp. Med, 174:1209-1220, 1991a. de Waal Malefyt, Haanen, Spits, Roncarolo, te Velde, Figdor, Johnson, Kastelein, Yssel, de Vries, "Interleukin 10 (rL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression," J. Exp. Med., 174:915-924, 1991b. de Waal Malefyt, Yssel, de Vries, "Direct effects of IL-10 on subsets of human CD4.sup.+ T cell clones and resting T cells," J. Immunol., 150:4754-4765, 1993. Del Prete, De Carli, Almerigigna, Giudizi, Biagiotti, Romagnani, "Human IL-10 is produced by both type 1 helper (Th1) and Type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production," J. Immunol., 150:353-360, 1993. Delvaux, Donckier, Bruyns, Florquin, Gerard, Amraoui, Abramowicz, "Effects of systemic administration of rIL-10 in an in vivo model of alloreactivity," Transplantation, 58:972-975, 1994. Ding, Linsley, Huang, Germain, Shevach, "IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression," J. Immunol., 151:1224-1234, 1993. Eissner, Lindner, Behrends, Kolch, Hieke, Klauke, Bornkamm, Holler, "Influence of bacterial endotoxin on radiation-induced activation of human endothelial cells in vitro and in vivo," Transplantation, 62:819-827, 1996. Enk, Angeloni, Udey, Katz, "Inhibition of Langerhans cell antigen-presenting function by IL-10," J. Immunol., 151:2390-2398, 1993. Enk, Saloga, Becker, Mohamadzadeh, Knop, "Induction of hapten-specific tolerance by interleukin 10 in vivo," J. Exp. Med, 179:1397-1402,1994. Fei, Castle, Barrett, Kastelein, Dang, Mostmann, Moore, Howard, "Interleukin 10, a novel B cell stimulatory factor: unresponsiveness of X chromosome-linked immunodeficiency B cells," J. Exp. Med., 172:1625-1631, 1990. Ferguson, Dube, Griffith, "Regulation of contact hypersensitivity by interleukin 10," J. Exp. Med., 179:1597-1604, 1994. Finbloom and Winestock, "IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1.alpha. and STAT3 complexes in human T cells and monocytes," J. Immunol., 155:1079-1090, 1995. Fiorentino, Bond, Mosmann, "Two types of mouse helper T cells. IV. Th2 clones secrete a factor that inhibits cytokine production by TH1 clones," J. Exp. Med., 170:2081-2095, 1989. Fiorentino, Zlotnik, Vieira, Mosmann, Howard, Moore, O'Garra, "IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells," J. Immunol.,

146:3444-3451, 1991a. Fiorentino, Zlotnik, Mosmann, Howard, O'Garra, "IL-10 inhibits inhibits cytokine production by activated macrophages," J. Immunol., 147:3815-3822, 1991b. Fleming, McCaughan, Andrews, Nash, Mercer, "A homologue of interleukin-10 is encoded by the poxvirus orf virus," J. Virol., 71:4857-4861, 1997. Florence, Howard, Howard, Chapman, Lieberman, Perkinson, Marks, "Reduction in the incidence of early rejection in cadaveric renal allograft recipients treated with ATGAM induction and sequential mycophenolate mofetil", Transplant Proc., 29(1-2):313-4, 1997. Flores-Villanueva, Zheng, Strom, Stadecker, "Recombinant IL-10 and IL-10/FC treatment down-down-regulate egg antigen-specific delayed hypersensitivity reactions and egg granuloma formation in schistosomiasis," J. Immunol., 156:3315-3320, 1996. Gaber, First, Tesi, Gaston, Mendez, Mulloy, Light, Gaber, Squiers, Taylor, Neylan, Steiner, Steiner, Knechtle, Norman, Shihab, Basadonna, Brennan, Hodge, Kahan, Kahan, Steinberg, Woodle, Chan, Ham, Schroeder, et al., "Results of the double-blind, randomized, multicenter, phase III clinical trial of Thymoglobulin versus Atgam in the treatment of acute graft rejection episodes after renal transplantation.", Transplantation, 66(1):29-37, 1998. Garlisi, Pennline, Smith, Siegel, Umland, "Cytokine gene expression in mice undergoing chronic graft-versus-host disease," Molec. Immunol., 30:669-677, 1993. Gerritsen, Bozeman, Elbers, van de Kerkhof, "Dithranol embedded in crystaline monoglycerides combined with phototherapy (UVB): A A new approach in the treatment of psoriasis," Skin Pharmacol. Appl. Skin Physiol. 11(3): 133-139, 1998. Gesser, Leffers, Jinquan, Vestergaard, Kirstein, Sindet-Pedersen, Jensen, Thestrup-Pedersen, Larsen, "Identification of functional domains on human interleukin 10," Proc. Natl. Acad. Sci. USA, 94:14620-14625, 1997. Gibbs and Pennica, "CRF24: isolation of cDNA clones encoding the human and mouse proteins," Gene, 186:97-101, 1997. Giovarelli, Musiani, Modesti, Dellabona, Casorati, Allione, Consalvo, Cavallo, di Pierro, De Giovanni, Musso, Forni, "Local release of IL-10 by transfected mouse mammary adenocarcinoma cells does not suppress suppress but enhances antitumor reaction and elicits a strong cytotoxic lymphocyte and antibody-dependent immune memory," J. Immunol., 155:3112-3123, 1995. Gorczynski and Wojcik, "A role for nonspecific (cyclosporin A) or specific (monoclonal antibodies to ICAM-1, LFA, and IL-10) immunomodulation in the prolongation of skin allografts after antigen-specific pretransplant immunization or transfusion," J. Immunol., 152:2011-2019, 1994. Gorczynski, Hozumi, Wolf, Chen, "Interleukin 12 in combination with anti-interleukin 10 reverses graft prolongation after portal venous venous immunization," Transplantation, 60:1337-1341, 1995. Groux, Bigler, de Vries, Roncarolo, "Interleukin-10 induces a long-term antigen-specific anergic state in human CD4.sup.+ T cells," J. Exp. Med., 184:19-29, 1996. Groux, O'Garra, Bigler, Rouleau, Antonenko, de Vries, Roncarolo, "A CD4.sup.+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis," Nature, 16:737-742, 1997. Groux, Bigler, de Vries, Roncarolo, "Inhibitory and stimulatory effects of IL-10 on human CDS.sup.+ T cells," J. Immunol., 160:3188-3193, 1998. Hagenbaugh, Sharma, Dubinett, Wei, Aranda, Cheroutre, Fowell, Binder, Tsao, Locksley, Moore, Kronenberg, Kronenberg, "Altered immune responses in interleukin 10 transgenic mice," J. Exp. Med., 185:2101-2110, 1997. Ho, Liu, Khan, Hsu, Bazan, Moore, "A receptor for interleukin 10 is related to interferon receptors," Proc. Natl. Acad Sci. USA, 90:11267-11271, 1993. Ho, Wei, Mui, Miyajima, Moore, "Functional regions of the mouse interleukin-10 receptor cytoplasmic domain," Molec. Cell. Biol, 15:5043-5053, 1995. Howard, Muchamuel, Andrade, Menon, "Interleukin 10 protects mice from lethal endotoxemia," J. Exp. Med., 177:1205-1208, 1993. Hsu, de Waal Malefyt, Fiorentino, Dang, Vieira, de Vries, Spits, Mosmann, Moore, "Expression of Interleukin-10 activity by epstein-barr virus protein BCRF1," Science, 250:830-832, 1990. Ishida, Muchamuel, Sakaguchi, Andrade, Menon, Howard, "Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F.sub.1 mice," J. Exp. Med., 179:305-310, 1994. Jinquan, Larsen, Gesser, Matsushima, Thestrup-Pedersen, "Human IL-10 is a chemoattractant for CD8.sup.+ T lymphocytes and an inhibitor of IL-8-induced CD4.sup.+ T lymphocyte migration," J. Immunol., 151:4545-4551, 1993. Jurlander, Lai, Tan, Chou, Geisler, Schriber, Blumenson, Narula, Baumann, Caligiuri, "Characterization of interleukin-10 receptor expression on B-cell chronic lymphocytic leukemia cells," Blood, 89:4146-4152, 1997. Kasama, Strieter, Lukacs, Lincoln, Burdick, "Interleukin-10 expression and chemokine

regulation during the evolution of murine type II collagen-induced arthritis," J. Clin. Invest., 95:2868-2876, 1995. Katsikis, Chu, Brennan, Maini, Feldmann "Immunoregulatory role of interleukin 10 in rheumatoid arthritis," J. Exp. Med, 179:1517-1 527, 1994. Kaye, Hsu, Sauron, Jameson, Gascoigne, Hedrick, "Selective development of CD4.sup.+ T cells in transgenic mice expressing a class II MHC-restricted antigen receptor," Nature, 341:746-749, 1989. Kotenko, Izotova, Pollack, Muthukumaran, Paukku, Silvennoinen, Ihle, Pestka, "Other kinases can substitute for Jak2 in signal transduction by interferon-.gamma.," J. Biol. Chem., 271:17174-17182, 17182, 1996. Kotenko, Krause, Izotova, Pollack, Wu, Pestka, "Identification and functional characterization of a second chain of the interleukin-10 receptor complex," EMBO J., 16:1594-5903, 1997. Krenger, Snyder, Smith, Ferrara, "Effects of exogenous interleukin-10 in a murine model of graft-versus-host disease to minor histocompatibility antigens," Transplantation, 58:1251-1257, 1994. Kumar, Cahill, Kumar, Panigrahi, Seirka, Singleton, al-Abdullah, Laskow, "ATGAM versus OKT3 induction therapy in cadaveric kidney transplantation: patient and graft survival, CD3 subset, infection, and cost analysis", Transplant Proc., 30(4):1351-2, 1998. Lai, Ripperger, Morella, Jurlander, Hawley, Carson, Kordula, Caligiuri, Hawley, Fey, Baumann, "Receptors for interleukin (IL)-10 and IL-6-type cytokines use similar signaling mechanisms for inducing transcription through IL-6 response elements," J. Biol. Chem., 271:13968-13975, 1996. Larner, David, Feldman, Igarashi, Hackett, Webb, Sweitzer, Petricoin III, Finbloom, "Tyrosine phosphorylation of DNA binding proteins by multiple cytokines," Science, 261:1730-1733, 1993. Le Moine, Marchant, Durand, Ickx, Pradier, Belghiti, Abramowicz, Gelin, Goldman, Deviere, "Systemic release of interleukin-10 during orthotopic liver transplantation," Hepatology, 20:889-892, 1994. Lee, Wogensen, Shizuru, Oldstone, Sarvetnick, "Pancreatic islet production of murine interleukin-10 does not inhibit immune-mediated tissue destruction," J. Clin. Invest., 93:1332-1338, 1994. Lehmann, Seegert, Strehlow, Schindler, Lohmann-Matthes, Decker, "IL-10-induced factors belonging to the p91 family of proteins bind to 12FN-.gamma.-responsive promoter elements," J. Immunol., 153:165-172, 1994. Li, Elliott, Mosmann, "IL-10 inhibits cytokine production, vascular leakage, and swelling during T helper 1 cell-induced delayed-type hypersensitivity," J. Immunol., 153:3967-3978, 1994. Liao, "Non surgical therapy for patients with advanced non-small cell lung cancer," Respirology 3(3):151-157, 1998. Liu, Wei, Ho, de Waal Malefyt, Moore, "Expression cloning and characterization of a human IL-10'receptor," J. Immunol., 152:1821-1829, 1994. Liu, de Waal Malefyt, Briere, Parham, Bridon, Banchereau, Moore, Xu, "The EBV IL-10 homologue is a selective agonist with impaired binding to the IL-10. receptor," J. Immunol., 158:604-613, 1997. Louw, Van Rensburg, Moola, Kotze, Marks, "Helicobacter pylori eradication and ulcer healing with daily lansoprazole, plus 1 or 2 weeks co-therapy with amoxycillin and clarithromycin," Ailment Pharmacol. Ther. 12(9):881-885, 1998. Lutfalla, Gardiner, Uze, "A new member of the cytokine receptor gene family maps on chromosome 21 at less than 35 kb from IFNAR," Genomics, 16:366-373, 1992.

☐ 20. Document ID: US 6352982 B1

L9: Entry 20 of 32 File: USPT Mar 5, 2002

DOCUMENT-IDENTIFIER: US 6352982 B1
TITLE: 4,1-benzoxazepines, their analogues, and their use as somatostatin agonists

Brief Summary Text (300):
The compounds (I) of the present invention or salts thereof may be used in a wide

variety of prophylactic, diagnostic, and therapeutic treatments of mammals(for example, human, cattle, horse, dog, cat, monkey, mouse and rat, especially, human) with low toxicity and with less adverse reactions. The compounds (I) of the present invention or salts thereof inhibit or modulate production or secretion of a variety of hormones, growth factors and physiologically active substances of mammals. As said "hormones" are mentioned, for example, growth hormones (GH), thyroid stimulating hormones (TSH), prolactin, insulin and glucagon. As said "growth factors" are mentioned, for example, IGF-1. As said "physiologically active substances" are mentioned, for example, vasoactive intestinal polypeptide (VIP), gastcin, glucagon-like peptide-1, amylin, substance-P, CGRP, CCK(cholecystokinin) and amylase. And that said "physiologically active substance" includes cytokines such as interleukins and TNF-.alpha.. The compounds or salts thereof of this invention function through somatostatin receptors which couple to a variety of intracellular signal transduction systems. These systems include adenylyl cyclase, K.sup.+ channels, Ca.sup.2+ channels, protein phosphatases, phospholipaseC/IP3 (inositol 1,4,5-trisphosphate), MAP kinase, a Na.sup.+ /H.sup.+ exchanger, phospholipase A2, a transcription factor such as NF-.kappa.B. The compounds or salts salts thereof of this invention modulate directly or indirectly cell proliferation inhibitory action of somatostatin and modulate apoptosis induced or regulated by somatostatin. The compounds or salts thereof of this invention may be used in a variety of diseases associated with disorders of production or secretion of hormones, growth factors, and physiologically active substances, associated with disorders of intracellular signal transduction systems, or associated with disorders disorders of regulating cell proliferation. Preferably, the compounds or salts thereof of this invention may be useful (1) for drugs for treatment of for example, tumors such as acromegaly, TSH-producing tumors, nonsecretory (afunctional) hypophysial tumors, ectopic ACTH (adrenocorticotrophic hormone)-producing tumors, medullar thyroid carcinoma, VIP-producing tumors, glucagon-producing tumors, gastrin-producing tumors, insulinoma and cartinoid tumor, (2) for drugs for treatment of insulin-dependent and non-insulin dependent diabetes mellitus or a variety of diseases associated with them, for example, diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, Doan syndrome and orthostatic hypotension, (3) for drugs for improvement of hyperinsulinemia or for treatment of obesity, for example, by inhibition of appetite (4) for drug for treatment of, for example, acute pancreatitis, chronic pancreatitis, pancreatointestinal fisutula, hemorrhagic ulcer, peptic ulcer, gastritis and hyperchylia by inhibition or modulation of the exocrine secretion in the digestive tracts, (5) for drugs for improvement of various symptoms associated with the Helicobacter pylori infection, for example, inhibitors of gastrin hypersecretion, (6) for drugs for inhibition of amylase secretion associated with endoscopic cholangiopancreatography, and drugs for prognostic treatment of surgical operation of pancreas, (7) for drugs for treatment of, for example, diarrhea due to intestinal malabsorption, promotion of secretion or dyskinesia of the digestive tracts(for example, short bowel syndrome), diarrhea due to the drugs for cancer chemotherapy, diarrhea due to AIDS, diarrhea due to graft versus host reaction (GVHR) associated with bone marrow transplantation, diarrhea due to diabetes mellitus, diarrhea due to celiac plexus blocking, diarrhea due to systemic sclerosis and diarrhea due to eosinophilia, (8) for drugs for treatment of, for example, dumping syndrome, irritable bowel syndrome, Crohn disease and inflammatory bowel disease, (9) for drugs for treatment of, for example, various cancers and tumors of which growth is dependent on insulin or IGF-1 or the other growth factors and various tumors and cancers associated with disorders of regulating cell proliferation caused by the other reasons (for example, thyroid cancer, colorectal cancer, breast cancer, prostatic cancer, small cell lung cancer, non-small cell cancer, pancreatic cancer, stomach cancer, cholangiocarcinoma, hepatic cancer, vesical cancer, ovarian cancer, melanoma, osteosarcoma, chondrosarcoma, malignant pheochromocytoma, neuro-blastoma, brain tumors, thymoma, renal cancers), leukemia (for example, leukemia of basophilic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, Hodgkin disease, and non-Hodgkin lymphoma) (drugs for treatment of these cancers can be used for monotherapy or concomitant therapy with other anticancer drugs, for example,

tamoxifen, LHRH agonists, LHRH antagonists, interferon-.alpha., Interferon-.beta., interferon-.gamma. and interleukin-2), (10) for drugs for prevention and treatment of, for example, hypertrophic cardiomyopathy, arteriosclerosis, valvulopathy, myocardiac infarction (especially, myocardiac infarction post percutaneous transluminal coronary arterioplasty) and reangioplasty, (11) for drugs for treatment of hemorrhage of esophageal varicosis, cirrhosis and peripheral blood vessel disorders, (12) for drugs for treatment of, for example, diseases associated with general or local inflammation, for example, polyarteritis, rheumatoid arthritis, psoriasis, sunburn, eczema and allergy (for example, asthma, atopic dermatitis and allergic rhinitis) because they inhibit or modulate the secretion of physiologically active substances acting on the immune system (for example, Substance P, tachykinin and cytokines), (13) for drugs for treatment of, for example, dementia (for example, Alzheimer disease, Alzheimer-type senile dementia, vascular/multi-infarct dementia), headache, migraine, schizophrenia, epilepsy, depression, generalized anxiety disorder, sleep disorder, and multiple sclerosis, because they give influence on the production and secretion of nerve regulators, (14) for analgesic drugs, (15) for drugs for treatment of, for example, acute bacterial meningitis, acute virus encephalitis, adult respiratory distress syndrome (ARDS), bacterial pneumonia, severe systemic mycotic infection, tuberculosis, spinal damage, bone fracture, hepatic failure, pneumonia, alcoholic hepatitis, virus A hepatitis, virus B hepatitis, virus C hepatitis, AIDS infection, human papilloma virus infection, influenza infection, metastasis of cancer, multiple myeloma, osteomalacia, osteoporosis, bone Paget disease, reflux esophagitis, nephritis, renal failure, sepsis, septic shock, hypercalcemia, hypercholesterolemia, hypertriglyceridemia, hyperlipemia, systemic lupus erythematosus, transient ischemic attach and alcoholic hepatitis, (16) for cure of, for example, organ trasplant, burns, trauma, and alopecia, (17) ocular diseases for example glaucoma, (18) for imaging of tumors having somatostain receptor after introducing a radioactive substance (for example, .sup.123 I, .sup.125 I, .sup.111 In) to the compounds of the present invention either directly or via a proper spacer, and (19) targeting tumors with somatostatin receptors using the compounds in the present invention conjugated with anti-cancer drugs directly or using an appropriate spacer.

☐ 21. Document ID: US 5908861 A

L9: Entry 21 of 32 File: USPT Jun 1, 1999

DOCUMENT-IDENTIFIER: US 5908861 A
TITLE: Methods for treating inflammation and inflammatory disease using pADPRT inhibitors

Brief Summary Text (5):
Malignant growth and inflammatory processes share the activation of certain cellular signal transduction pathways, e.g., MAP kinase; Kyriakis et al., 1996, "Sounding the alarm: protein kinase cascades activated by stress and inflammation," J. Biol Chem. 271:24313-24316; Ferrell, J E, 1996, "Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs," TIBS 21:460-466. Chronic inflammation frequently leads to carcinogenic transformation, as demonstrated, for example, in the case of the intestinal epithelium; Kawai et al., 1993, "Enhancement of rat urinary bladder tumorigenesis by lipopolysaccharide-induced inflammation," Cancer Res. 53:5172-5; Rosin et al., 1994, "Inflammation, chromosomal instability, and cancer: the schistosomiasis

model," Cancer Res. 54 (7 Suppl):1929s-1933s; Choi et al., 1994, "Similarity of colorectal cancer in Crohn's disease and ulcerative colitis: implications for carcinogenesis and prevention," Gut 35:950-4. Based on the connection between chronic inflammation and carcinogenic transformation, the aim of the present study was to investigate whether INH.sub.2 BP affects the course of the inflammatory process in vitro and in vivo. In our study, the production of multiple proinflammatory mediators was induced by bacterial lipopolysaccharide (endotoxin, LPS). LPS is known to induce a multitude of cellular reactions and triggers a systemic inflammatory response. LPS-induced pro-inflammatory mediators include tumor tumor necrosis factor alpha (TNF), interleukin-1, interferon-gamma, whereas antiinflammatory mediators include interleukin-10 (IL-10) and interleukin-13; Deltenre et al., 1995, "Gastric carcinoma: the Helicobacter pylori trail," Acta Gastroenterol Belg. 58:193-200; Beutler, 1995, "TNF, immunity and inflammatory disease: lessons of the past decade," J. Invest. Med. 42:227-35; Liles et al., 1995, "Review: nomenclature and biologic significance of cytokines involved in inflammation and the host immune response," J. Infect Dis. 172:1573-80; Giroir, 1993, "Mediators of septic shock: new approaches for interrupting the endogenous inflammatory cascade," Critical Car. Med. 21:780-9. As a consequence of the production of these inflammatory cytokines, LPS initiates the production of inflammatory free radicals (oxygen-centered, such as superoxide, and nitrogen-centered radicals, such as nitric oxide [NO]) and of prostaglandins; Nathan, 1992, "Nitric oxide as a secretory product of mammalian cells," FASEB J. 6:3051-3064; Vane, J. R., The Croonian Lecture 1993, "The endothelium: maestro of the blood circulation," Proc. Roy. Soc. Lond B 343:225-246; Szabo, C.; 1995, "Alterations in the production of nitric oxide in various forms of circulatory shock," New Horizons 3:3-32. The production of NO in inflammation is due to the expression of a distinct isoform of NO synthase (iNOS), while the production of inflammatory cytokines is explained by the expression of a distinct isoform of cyclooxygenase (cyclooxygenase-2, COX-2); Nathan, 1992, "Nitric oxide as a secretory product of mammalian cells," FASEB J. 6:3051-3064; Vane, J. R., The Croonian Lecture 1993, "The endothelium: maestro of the blood circulation," Proc. Roy. Soc. Lond B 343:225-246; Szabo, C.; 1995, "Alterations in the production of nitric oxide in various forms of circulatory shock," New Horizons 3:3-32. iNOS, COX-2, as well as the above mentioned pro-inflammatory cytokines and free radicals which play an important role in the LPS-induced inflammatory response; ; Nathan, 1992, "Nitric oxide as a secretory product of mammalian cells," FASEB J. 6:3051-3064; Vane, J. R., The Croonian Lecture 1993, "The endothelium: maestro of the blood circulation," Proc. Roy. Soc. Lond B 343:225-246; Szabo, C.; 1995, "Alterations in the production of nitric oxide in various forms of circulatory shock," New Horizons 3:3-32. Moreover, NO (or its toxic byproduct, peroxynitrite), has been implicated as a key mediator leading to the transformation of the inflammatory response into a carcinogenic process; Bartsch et al., 1994, "Endogenously formed N-nitroso compounds and nitrosating agents in human cancer etiology," Pharmacogenetics 2:272-7; Liu et al., 1992, "Woodchuck hepatitis virus surface antigen induces NO synthesis in hepatocytes: possible role in hepatocarcinogenesis.," Carcinogenesis 15:2875-7; Ohshima et al., 1994, "Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis," Mutation Res. 305:253-64. In the current studies, we have first investigated whether treatment with INH.sub.2 BP affects the production of the inflammatory mediators tumor necrosis factor alpha [TNF], interleukin-10, interleukin-6, NO, and prostaglandin in vivo, in LPS-induced models of inflammation.

☐ 22. Document ID: AU 2003303345 A1, WO 2004058718 A1, US 20040192612 A1

L9: Entry 22 of 32 File: DWPI Jul 22, 2004

DERWENT-ACC-NO: 2004-534086
DERWENT-WEEK: 200476
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New 4-oxo-3(1-oxo-1H-isoquinolin-2-ylacetylamino)-pentanoic acid esters and amide derivatives are caspase inhibitors, useful to treat e.g. interleukin mediated diseases, apoptosis mediated diseases and inflammatory diseases

INVENTOR: CHARRIER, J; MORTIMORE, M ; STUDLEY, J R

PRIORITY-DATA: 2002US-435133P (December 20, 2002), 2003US-0743563 (December 22, 2003)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 2003303345 A1	July 22, 2004		000	C07D217/24
WO 2004058718 A1	July 15, 2004	E	104	C07D217/24
US 20040192612 A1	September 30, 2004		000	A61K031/47

INT-CL (IPC): A61 K 31/47; A61 K 31/472; A61 K 31/4725; C07 D 217/22; C07 D 217/24; C07 D 401/12; C07 D 405/12; C07 D 417/12

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 23. Document ID: AU 2003258366 A1, WO 2004022727 A1

L9: Entry 23 of 32 File: DWPI Mar 29, 2004

DERWENT-ACC-NO: 2004-248465
DERWENT-WEEK: 200459
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Novel Lactobacillus fermentum variant that ferments ribose or galactose and propagates in bile salts, useful for preventing and/or treating gastrointestinal disorder e.g. irritable bowel syndrome, diarrhea

INVENTOR: CONWAY, P L

PRIORITY-DATA: 2002AU-0951270 (September 6, 2002)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 2003258366 A1	March 29, 2004		000	C12N001/20
WO 2004022727 A1	March 18, 2004	E	063	C12N001/20

INT-CL (IPC): C12 N 1/20

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 24. Document ID: EP 1485107 A1, WO 2003068242 A1, US 20040019017 A1, AU 2003211052 A1

L9: Entry 24 of 32 File: DWPI Dec 15, 2004

DERWENT-ACC-NO: 2003-697485
DERWENT-WEEK: 200482
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New compound having phospholipid moiety covalently linked by a bridging group group useful in the treatment of e.g. autoimmune disease, inflammatory disease, proliferative disorder, degenerative disease and uveitis

INVENTOR: GOLEC, J M C; MORTIMORE, M

PRIORITY-DATA: 2002US-355889P (February 11, 2002), 2003US-0366192 (February 11, 2003)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
EP 1485107 A1	December 15, 2004	E	000	A61K031/685
WO 2003068242 A1	August 21, 2003	E	128	A61K031/685
US 20040019017 A1	January 29, 2004		000	A61K031/685
AU 2003211052 A1	September 4, 2003		000	A61K031/685

INT-CL (IPC): A61 K 31/66; A61 K 31/685; A61 P 37/06; A61 P 37/066; C07 C 237/36; C07 C 237/366; C07 C 237/40; C07 C 237/400; C07 D 209/26; C07 D 209/266; C07 D 209/86; C07 D 209/866; C07 D 209/94; C07 D 209/944; C07 D 211/34; C07 D 211/344; C07 D 239/90; C07 D 239/900; C07 D 271/06; C07 D 271/066; C07 D 409/06; C07 D 409/066; C07 D 413/12; C07 D 413/122; C07 D 417/06; C07 D 417/066; C07 D 471/04; C07 D 471/044; C07 F 9/10; C07 F 9/100; C07 F 9/28; C07 K 5/02; C07 K 5/022; C07 K 5/06; C07 K 5/066

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw D

☐ 25. Document ID: EP 1476461 A2, WO 2003068812 A2, DE 10207734 A1, DE 10240866 A1, AU 2003206900 A1

L9: Entry 25 of 32 File: DWPI Nov 17, 2004

DERWENT-ACC-NO: 2003-646480
DERWENT-WEEK: 200475
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New polypeptide that binds immunoglobulin E and alters cytokine synthesis, useful for treating e.g. atopic eczema, asthma or allergy, also its encoding nucleic acid

INVENTOR: NEUBER, K

PRIORITY-DATA: 2002DE-1040866 (September 4, 2002), 2002DE-1007734 (February 15, 2002)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
EP 1476461 A2	November 17, 2004	G	000	C07K014/31
WO 2003068812 A2	August 21, 2003	G	046	C07K014/31
DE 10207734 A1	September 4, 2003		000	C07K007/06
DE 10240866 A1	March 18, 2004		000	C07K014/195
AU 2003206900 A1	September 4, 2003		000	C07K014/31

INT-CL (IPC): A61 K 38/00; A61 K 38/000; A61 K 38/08; A61 K 38/16; A61 K 39/085; A61 K 39/0855; C07 K 7/06; C07 K 14/195 ; C07 K 14/31; C12 N 15/12; C12 N 15/31; C12 N 15/311

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. De

☐ 26. Document ID: MX 2003009647 A1, WO 200285899 A1, US 20030096737 A1, EP 1381602 A1, AU 2002254693 A1, JP 2004527548 W

L9: Entry 26 of 32 File: DWPI Feb 1, 2004

DERWENT-ACC-NO: 2003-093086
DERWENT-WEEK: 200473
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New heterocyclyldicarbamides useful for treating or preventing TNF-alpha mediated disease

INVENTOR: DIU-HERCEND, A; GOLEC, J ; HERCEND, T ; KNEGTEL, R ; LANG, P ; MILLER, A ; MILLER, K ; MORTIMORE, M ; WEBER, P ; DIUHERCEND, A ; MARTIMORE, M

PRIORITY-DATA: 2001US-285051P (April 19, 2001), 2002US-0127324 (April 19, 2002)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
MX 2003009647 A1	February 1, 2004		000	A61K031/445
WO 200285899 A1	October 31, 2002	E	094	C07D417/06
US 20030096737 A1	May 22, 2003		000	A61K038/05
EP 1381602 A1	January 21, 2004	E	000	C07D417/06
AU 2002254693 A1	November 5, 2002		000	C07D417/06
JP 2004527548 W	September 9, 2004		156	C07D211/60

INT-CL (IPC): A61 K 31/445; A61 K 31/4525; A61 K 31/4535; A61 K 31/454; A61 K 31/4545; A61 K 31/4725; A61 K 38/05; A61 P 1/02; A61 P 1/04; A61 P 1/16; A61 P 1/18; A61 P 3/10; A61 P 5/14; A61 P 7/00; A61 P 7/06; A61 P 9/00 ; A61 P 9/10; A61 P 11/00; A61 P 11/02; A61 P 11/06; A61 P 13/12; A61 P 17/00; A61 P 17/02; A61 P 17/04; A61 P 17/06; A61 P 17/14; A61 P 19/00; A61 P 19/02; A61 P 19/06; A61 P 19/10; A61 P 21/00; A61 P 21/04; A61 P 25/00; A61 P 25/08; A61 P 25/14; A61 P 25/16; A61 P 25/28; A61 P 25/32; A61 P 27/02; A61 P 27/14; A61 P 29/00; A61 P 31/00; A61 P 31/04; A61 P 31/06; A61 P 31/12; A61 P 31/14; A61 P 31/18; A61 P 31/20; A61 P 35/00; A61 P 35/02; A61 P 37/00; A61 P 37/02; A61 P 37/06; A61 P 37/08; C07 D 211/06; C07 D 211/60; C07 D 241/04; C07 D 265/30; C07 D 279/12; C07 D 401/06; C07 D 405/06; C07 D 409/06; C07 D 417/06; C07 K 5/04

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. De

☐ 27. Document ID: US 20040038329 A1, WO 200203065 A1, AU 200168835 A, EP 1299721 A1, BR 200112155 A, KR 2003021235 A, CN 1444732 A, JP 2004502186 W

L9: Entry 27 of 32 File: DWPI Feb 26, 2004

DERWENT-ACC-NO: 2002-154775
DERWENT-WEEK: 200416

COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Monitoring progress of treatment and/or eradication of Helicobacter pylori infection in a subject undergoing treatment by determining immunoglobulin G2 anti-H.pylori antibody level in saliva sample

INVENTOR: BORODY, T J; CLANCY, R L ; PANG, G ; REN, Z

PRIORITY-DATA: 2000AU-0008541 (July 3, 2000)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20040038329 A1	February 26, 2004		000	C12Q001/24
WO 200203065 A1	January 10, 2002	E	033	G01N033/53
AU 200168835 A	January 14, 2002		000	G01N033/53
EP 1299721 A1	April 9, 2003	E	000	G01N033/53
BR 200112155 A	June 10, 2003		000	G01N033/53
KR 2003021235 A	March 12, 2003		000	G01N033/53
CN 1444732 A	September 24, 2003		000	G01N033/53
JP 2004502186 W	January 22, 2004		050	G01N033/53

INT-CL (IPC): C12 Q 1/04; C12 Q 1/24; G01 N 33/50; G01 N 33/53; G01 N 33/569

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 28. Document ID: NZ 519424 A, WO 200142216 A2, AU 200124283 A, BR 200016282 A, EP 1244626 A2, NO 200202656 A, CZ 200201970 A3, KR 2002060259 A, SK 200200807 A3, JP 2003516393 W, CN 1420872 A, ZA 200204390 A, HU 200300782 A2

L9: Entry 28 of 32 File: DWPI Mar 26, 2004

DERWENT-ACC-NO: 2001-457279
DERWENT-WEEK: 200425
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TITLE: New heterocyclic amide derivatives useful as caspase inhibitors for treating e.g. Alzheimer's disease, leukemia, diabetes, osteoporosis, sepsis, burns, organ transplant rejection and congestive heart failure

INVENTOR: BINCH, H; CHARIFSON, P ; CHARRIER, J ; GOLEC, J ; CHARIFSON, P S ; GOLEC, J M C

PRIORITY-DATA: 1999US-169812P (December 8, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
NZ 519424 A	March 26, 2004		000	C07D213/64
WO 200142216 A2	June 14, 2001	E	088	C07D213/64
AU 200124283 A	June 18, 2001		000	C07D213/64
BR 200016282 A	August 27, 2002		000	C07D213/64
EP 1244626 A2	October 2, 2002	E	000	C07D213/64
NO 200202656 A	August 6, 2002		000	C07D213/64
CZ 200201970 A3	October 16, 2002		000	C07D213/64

KR 2002060259 A	July 16, 2002	000	C07D213/64
SK 200200807 A3	February 4, 2003	000	C07D213/64
JP 2003516393 W	May 13, 2003	112	C07D209/46
CN 1420872 A	May 28, 2003	000	C07D213/64
ZA 200204390 A	August 27, 2003	137	C07D000/00
HU 200300782 A2	September 29, 2003	000	C07D213/64

INT-CL (IPC): A61 K 31/403; A61 K 31/4035; A61 K 31/4375; A61 K 31/4412; A61 K 31/47; A61 K 31/4704; A61 K 31/472; A61 K 31/513; A61 K 31/517; A61 K 31/519; A61 P 1/00; A61 P 1/04; A61 P 1/16; A61 P 1/18; A61 P 3/10; A61 P 5/14; A61 P 7/00; A61 P 7/06; A61 P 9/00; A61 P 9/04; A61 P 9/10; A61 P 11/00; A61 P 11/06; A61 P 13/12; A61 P 17/00; A61 P 17/02; A61 P 17/06; A61 P 17/14; A61 P 19/00; A61 P 19/02; A61 P 19/08; A61 P 19/10; A61 P 21/00; A61 P 21/04; A61 P 25/00; A61 P 25/14; A61 P 25/16; A61 P 25/28; A61 P 25/32; A61 P 27/02; A61 P 29/00; A61 P 31/00; A61 P 31/04; A61 P 31/12; A61 P 31/18; A61 P 35/00; A61 P 35/02; A61 P 37/02; C07 D 0/00; C07 D 209/44; C07 D 209/46; C07 D 209/48; C07 D 213/64; C07 D 215/22; C07 D 217/24; C07 D 239/36; C07 D 239/80; C07 D 239/90; C07 D 471/04; C07 D 495/04; C07 M 7:00

Full | Title | Citation | Front | Review | Classification | Date | Reference | [redacted] | Claims | KWMC | Draw. D

☐ 29. Document ID: JP 2001524603 X, WO 200121177 A1, JP 2001158740 A, AU 200073159 A

L9: Entry 29 of 32 File: DWPI Apr 8, 2003

DERWENT-ACC-NO: 2001-281577
DERWENT-WEEK: 200333
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Chemokine/cytokine expression inhibitors comprise triazolopyridazine compounds

INVENTOR: ISAKA, M; KAWANO, Y ; MATSUMOTO, T

PRIORITY-DATA: 1999JP-0266279 (September 20, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 2001524603 X	April 8, 2003		000	A61K031/5025
WO 200121177 A1	March 29, 2001	J	052	A61K031/5025
JP 2001158740 A	June 12, 2001		017	A61K031/5025
AU 200073159 A	April 24, 2001		000	A61K031/5025

INT-CL (IPC): A61 K 31/5025; A61 P 1/00; A61 P 1/04; A61 P 1/16; A61 P 1/18; A61 P 3/02; A61 P 3/06; A61 P 3/10; A61 P 7/04; A61 P 9/00; A61 P 9/10; A61 P 11/06; A61 P 13/12; A61 P 19/00; A61 P 19/02; A61 P 19/08; A61 P 19/10; A61 P 25/00; A61 P 25/08; A61 P 25/28; A61 P 27/02; A61 P 29/00; A61 P 31/04; A61 P 31/06; A61 P 31/12; A61 P 31/18; A61 P 35/00; A61 P 35/02; A61 P 37/02; A61 P 37/08; A61 P 43/00; C07 D 487/04

Full | Title | Citation | Front | Review | Classification | Date | Reference | [redacted] | Claims | KWMC | Draw. D

☐ 30. Document ID: MX 2001011721 A1, WO 200070348 A1, AU 200043862 A, BR 200010558 A, EP 1183540 A1, CN 1355885 A, KR 2002033093 A, JP 2002544520 W, US 20040157277 A1

L9: Entry 30 of 32 File: DWPI Sep 1, 2003

DERWENT-ACC-NO: 2001-025046
DERWENT-WEEK: 200465

TITLE: Diagnosis of gastric cancer, or assessment of the risk of developing it, using a largely non-invasive method which uses the level of e.g., gamma-interferon or interleukin-4, as a marker

INVENTOR: CLANCY, R L; PANG, G

PRIORITY-DATA: 1999AU-0000377 (May 14, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
MX 2001011721 A1	September 1, 2003		000	C12Q001/68
WO 200070348 A1	November 23, 2000	E	027	G01N033/574
AU 200043862 A	December 5, 2000		000	G01N033/574
BR 200010558 A	February 19, 2002		000	G01N033/574
EP 1183540 A1	March 6, 2002	E	000	G01N033/574
CN 1355885 A	June 26, 2002		000	G01N033/574
KR 2002033093 A	May 4, 2002		000	G01N033/574
JP 2002544520 W	December 24, 2002		024	G01N033/574
US 20040157277 A1	August 12, 2004		000	G01N033/574

INT-CL (IPC): C12 Q 1/02; C12 Q 1/68; G01 N 33/53; G01 N 33/554; G01 N 33/569; G01 N 33/574

☐ 31. Document ID: JP 08073453 A, US 6034100 A

L9: Entry 31 of 32 File: DWPI Mar 19, 1996

DERWENT-ACC-NO: 1996-205508
DERWENT-WEEK: 200019

TITLE: TNF-alpha, interleukin-6 or -8 or interferon-gamma inhibitor - comprises 4-quinolone-3-carboxylic acid deriv., useful e.g. for treating rheumatoid arthritis or endotoxin shock

INVENTOR: ADACHI, M; OMORI, K ; ONO, Y ; TAMAOKA, H

PRIORITY-DATA: 1994JP-0209518 (September 2, 1994), 1993JP-0048501 (March 10, 1993)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 08073453 A	March 19, 1996		006	C07D401/04

US 6034100 A March 7, 2000 000 A61K031/47

INT-CL (IPC): A61 K 31/47; A61 K 31/495; C07 D 401/04

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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☐ 32. Document ID: WO 9420105 A1, MX 197232 B, AU 9460461 A, JP 06316521 A, TW 244336 A, EP 688218 A1, AU 676267 B, CN 1118991 A, JP 2694321 B2, EP 688218 B1, DE 69421694 E, ES 2140531 T3, US 6034100 A, KR 191943 B1

L9: Entry 32 of 32 File: DWPI Sep 15, 1994

DERWENT-ACC-NO: 1994-302661
DERWENT-WEEK: 200133

TITLE: New interleukin-1-inhibiting agents - having a benzoheterocyclic cpd. as active ingredient

INVENTOR: ADACHI, M; ONO, Y ; TAMAOKA, H ; OMORI, K

PRIORITY-DATA: 1993JP-0048501 (March 10, 1993), 1994JP-0209518 (September 2, 1994)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9420105 A1	September 15, 1994		021	A61K031/47
MX 197232 B	June 28, 2000		000	A61K031/047
AU 9460461 A	September 26, 1994		000	A61K031/47
JP 06316521 A	November 15, 1994		006	A61K031/47
TW 244336 A	April 1, 1995		000	A61K031/47
EP 688218 A1	December 27, 1995	E	000	A61K031/47
AU 676267 B	March 6, 1997		000	A61K031/47
CN 1118991 A	March 20, 1996		000	A61K031/47
JP 2694321 B2	December 24, 1997		006	A61K031/47
EP 688218 B1	November 17, 1999	E	000	A61K031/47
DE 69421694 E	December 23, 1999		000	A61K031/47
ES 2140531 T3	March 1, 2000		000	A61K031/47
US 6034100 A	March 7, 2000		000	A61K031/47
KR 191943 B1	June 15, 1999		000	A61K031/47

INT-CL (IPC): A61 K 31/047; A61 K 31/47; A61 K 31/495; C07 D 401/04; C07 D 455/04

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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Terms	Documents

L8 same L4	32

Display Format: - Change Format

Previous Page Next Page Go to Doc#

L9: Entry 6 of 32 File: PGPB Mar 25, 2004

DOCUMENT-IDENTIFIER: US 20040057926 A1
TITLE: Modulation of the immune response through the manipulation of arginine levels levels

Detail Description Paragraph:
[0283] H. pylori infection induces an inflammatory response characterized by infiltrating polymorphonuclear leukocytes, macrophages and lymphocytes, and the production of several inflammatory cytokines including TNF-60 , IFN-.gamma. and IL8. Tanahashi, T., et al. Infect. Immun. 68, pp. 664-671 (2000); Bauditz, J., et al. Clin. Exp. Immunol. 117, pp. 316-323 (1999); Beales, I. L., et al. Cytokine 9, pp. 514-520 (1997); Sharma, S. A., et al. J. Immunol. 160, pp. 2401-2407 (1998); Yamada, H., et al. Biochem. Pharmacol. 61, pp. 1595-1604 (2001). However, this strong immune response appears to confer little or no protection against H. pylori infection. In vitro models show that virulent strains of H. pylori (carrying the PAI) can impair phagocytosis by delaying actin rearrangement. Allen, L. A., et al. J. Exp. Med. 191, pp. 115-128 (2000). Once phagocytosed, these strains of H. pylori cause the fusion of phagosomes into megasomes, decreasing the killing ability of macrophages. Allen, L. A., et al. J. Exp. Med. 191, pp. 115-128 (2000). In doing so, H. pylori not only delays its own phagocytosis, but also that of other particles particles and bacteria. Ramarao, N., et al. Infect. Immun. 69, pp. 2604-2611 (2001). However, little is known on how H. pylori affects T-cells.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU00/00441

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	96/12965	AU	38143/95
WO	98/24885	IT	1289578

END OF ANNEX

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00441

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: G01N 33/574, G01N 33/569, C12Q 1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Derwent WPAT, JAPIO; STN MEDLINE CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Journal of Gastroenterology, vol. 31(4), 485-490; Ishihara, S. et al. (1996) Cytokine gene expression in the gastric mucosa: Its role in chronic gastritis. See the introduction, tables, figures and discussion in particular.	2-16, 19-21 1-22
X Y	Immunology Letters, vol. 48(1), 45-8; Fan, X. et al. (1995) Effect of IL-4 on peripheral blood lymphocyte proliferation: implication in immunopathogenesis of H.pylori infection. See the introduction and discussion in particular.	2-22 1-22
X Y	Infection and Immunity, vol.67 (1), 279-85; Sawai, N. et al. (1999) Role of Gamma Interferon in Helicobacter pylori-Induced Gastric Inflammatory Responses in a Mouse Model. See the abstract and the conclusion in particular.	2, 4-22 1-22

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 2000	04 JUL 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **DAVID HENNESSY** Telephone No : (02) 6283 2255

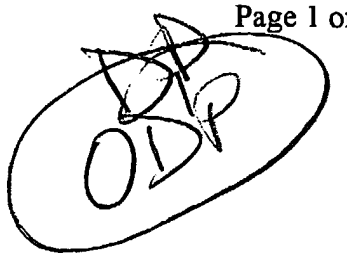
Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/00441

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Italian Journal of Gastroenterology and Hepatology, vol. 31(5), 408-15; Zagari, R.M. et al. (1999) Review article: non-invasive methods for the diagnosis of Helicobacter pylori infection. See serology, references 7,8 in particular.	1-22
X Y	The Lancet, vol. 347, 269-270; Liston, R. et al. (1996) IgG ELISA antibodies and detection of Helicobacter pylori in elderly patients. See both letters.	1, 6-22 2-5
X Y	Journal of Clinical Pathology, vol. 49, 112-115; Osawa, H. et al. (1996) Inverse relation of serum Helicobacter pylori antibody titres and extent of intestinal metaplasia. See the whole article.	1,6-22 2-5
X Y	Gut, vol. 36, 341-5; Karttunen, R. et al. (1995) Interferon gamma and interleukin 4 secreting cells in the gastric antrum in Helicobacter pylori positive and negative gastritis. See pages 341 and 344 in particular.	2-22 1
T	Journal of Gastroenterology, vol. 34, 560-570; Itoh, T. et al. (1999) The vast majority of gastric T cells are polarized to produce T helper 1 type cytokines upon antigenic stimulation despite the absence of Helicobacter pylori infection.	1-22
A	WO 96/12965 A1 (Genelabs Diagnostics Pty Ltd) 02.05.96; see 'Background of the Invention', example 2 and claim 1 in particular.	1-22
Y	WO 98/24885 A1 (Sanitaria Scaligera S.P.A.) 11.06.98; See the examples in particular.	1-22



L5: Entry 3 of 9 File: PGPB Feb 26, 2004

PGPUB-DOCUMENT-NUMBER: 20040038329
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040038329 A1

TITLE: Methods for monitoring treatment of helicobacter infection and for predicting predicting the likelihood of successful eradication

PUBLICATION-DATE: February 26, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Clancy, Robert Llewellyn	New South Wales		AU	
Borody, Thomas Julius	New South Wales		AU	
Pang, Gerarld	New South Wales		AU	
Ren, Zhigang	New South Wales		AU	

APPL-NO: 10/ 332112 [PALM]
DATE FILED: July 31, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
AU	PQ 8541	2000AU-PQ 8541	July 3, 2000

PCT-DATA:

DATE-FILED	APPL-NO	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
Jul 3, 2001	PCT/AU01/00795				

INT-CL: [07] C12 Q 1/24, C12 Q 1/04

US-CL-PUBLISHED: 435/30; 424/164.1, 424/234.1, 424/93.4, 435/34
US-CL-CURRENT: 435/30; 424/164.1, 424/234.1, 424/93.4, 435/34

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The present invention relates to methods for monitoring treatment of Helicobacter infection and in particular to methods for monitoring eradication of Helicobacter pylori infection using immunoglobulin G2 (IgG2). The invention also relates to methods for predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection and in particular, to methods for predicting the likelihood of successful eradication including determining the levels of interleukin, interferon-.gamma. and IgG in the subject to be, or being treated.

L5: Entry 3 of 9 File: PGPB Feb 26, 2004

PGPUB-DOCUMENT-NUMBER: 20040038329
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040038329 A1

TITLE: Methods for monitoring treatment of helicobacter infection and for predicting predicting the likelihood of successful eradication

PUBLICATION-DATE: February 26, 2004

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/30; 424/164.1, 424/234.1, 424/93.4, 435/34

CLAIMS:

The claims defining the invention are as follows:

1. A method of monitoring eradication of Helicobacter infection in a subject treated for the infection, including: i) determination of IgG2 anti-H. pylori antibody level in a saliva sample; ii) comparison of the IgG2 anti-H. pylori antibody level with a predetermined control IgG2 anti-H. pylori antibody level, wherein a reduction in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates eradication of Helicobacter.

2. A method of monitoring efficacy of treatment of Helicobacter infection in a subject treated for the infection, including: i) determination of IgG2 anti-H. pylori antibody level in a saliva sample; ii) comparison of the IgG2 anti-H. pylori antibody level with a predetermined control IgG2 anti-H. pylori antibody level, wherein a reduction in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates efficacious treatment of Helicobacter.

3. A method of monitoring relapse or reinfection with Helicobacter in a subject treated for infection with Helicobacter, including: i) determination of IgG2 anti-H. H. pylori antibody level in a saliva sample; ii) comparison of the IgG2 anti-H. pylori antibody level with a predetermined control IgG2 anti-H. pylori antibody level, wherein an increase in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates relapse or reinfection with Helicobacter.

4. A method of detecting unresponsiveness of a subject to treatment of Helicobacter infection, including: (i) determination of IgG2 anti-H. pylori antibody level in a saliva sample; (ii) comparison of the IgG2 anti-H. pylori antibody level with a

predetermined control IgG2 anti-H. pylori antibody level, wherein lack of change in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates lack of response to treatment.

5. A method according to any one of claims 1 to 4, wherein the IgG2 anti-H. pylori antibody is detected by an immunoassay.

6. A method according to claim 5, wherein the assay is ELISA.

7. A method according to any one of claims 1 to 6, wherein the control levels of IgG2 anti-H. pylori antibody is established in samples of saliva obtained from subjects not infected by H. pylori.

8. A method according to any one of claims 1 to 6, wherein the control levels of IgG2 anti-H. pylori antibody are determined in subject's own saliva sample.

9. A kit for monitoring treatment of Helicobacter infection, including, (i) Helicobacter antigen (ii) reagent for determining IgG2 subclass antibody.

10. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination of IL-4 level in a sample from the subject; (ii) comparison of the IL-4 level with a predetermined control or standard IL-4 level, (iii) wherein a level of IL-4 in the sample from the subject above the control or standard IL-4 level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard IL-4 level is predictive of the likelihood of eradication failure.

11. A method according to claim 10 wherein the sample is a blood sample.

12. A method according to claim 10 or claim 11, wherein the IL-4 is detected by an immunoassay.

13. A method according to claim 12, wherein the assay is ELISA.

14. A method according to any one of claims 10 to 13, wherein the control or standard level of IL-4 is established from analysis of samples obtained from subjects not infected by H. pylori and/or subjects having successfully eradicated H. pylori and/or subjects infected by H. pylori.

15. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination of interferon-.gamma. (INF-.gamma.) level in a sample from the subject; (ii) comparison of the INF-.gamma. level with a predetermined control or standard INF-.gamma. level, (iii) wherein a level of INF-.gamma. in the sample from the subject below the control or standard INF-.gamma. level is predictive of the likelihood of successful eradication and a level of INF-.gamma. above the control or standard level is predictive of the likelihood of eradication failure.

16. A method according to claim 15 wherein the sample is a blood sample.

17. A method according to claim 15 or claim 16, wherein the IFN-.gamma. level is detected by an immunoassay.

18. A method according to claim 17, wherein the assay is ELISA.

19. A method according to any one of claims 15 to 18, wherein the control or standard level of IFN-.gamma. is established from analysis of samples obtained from subjects not infected by H. pylorii and/or subjects having successfully eradicated H. pylori. and/or subjects infected by H. pylori.

20. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination of immunoglobulin G (IgG) level in a sample from the subject; (ii) (ii) comparison of the IgG level with a predetermined control or standard IgG level, level, (iii) wherein a level of IgG in the sample from the subject below the control control or standard level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

21. A method according to claim 20 wherein the sample is a serum sample.

22. A method according to claim 20 wherein the sample is a saliva sample.

23. A method according to any one of claims 20 to 22, wherein the control or standard level of IgG is established from analysis of samples obtained from subjects subjects not infected by H. pylori and/or subjects having successfully eradicated H. H. pylori and/or subjects infected by H. pylori.

24. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including: (i) determination a combination of IL-4 and/or INF-.gamma. and/or IgG levels in a sample from the subject; (ii) comparison of the IL-4 and/or INF-.gamma. and/or IgG levels with a predetermined control or standard L-4 and/or IF-.gamma. and/or IgG level respectively, wherein a level of IL-4 in the sample from the subject above the the control or standard level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard level is predictive of of the likelihood of eradication failure, and wherein a level of INF-.gamma. in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IFN-.gamma. above the control or or standard level is predictive of the likelihood of eradication failure, and wherein a level of IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

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L5: Entry 8 of 9 File: DWPI Feb 26, 2004

DERWENT-ACC-NO: 2002-154775
DERWENT-WEEK: 200416

TITLE: Monitoring progress of treatment and/or eradication of Helicobacter pylori infection in a subject undergoing treatment by determining immunoglobulin G2 anti-H.pylori antibody level in saliva sample

INVENTOR: BORODY, T J; CLANCY, R L ; PANG, G ; REN, Z

PRIORITY-DATA: 2000AU-0008541 (July 3, 2000)

PATENT-FAMILY:

	PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
☐	US 20040038329 A1	February 26, 2004		000	C12Q001/24
☐	WO 200203065 A1	January 10, 2002	E	033	G01N033/53
☐	AU 200168835 A	January 14, 2002		000	G01N033/53
☐	EP 1299721 A1	April 9, 2003	E	000	G01N033/53
☐	BR 200112155 A	June 10, 2003		000	G01N033/53
☐	KR 2003021235 A	March 12, 2003		000	G01N033/53
☐	CN 1444732 A	September 24, 2003		000	G01N033/53
☐	JP 2004502186 W	January 22, 2004		050	G01N033/53

INT-CL (IPC): C12 Q 1/04; C12 Q 1/24; G01 N 33/50; G01 N 33/53; G01 N 33/569

ABSTRACTED-PUB-NO: WO 200203065A
BASIC-ABSTRACT:

NOVELTY - Monitoring eradication of Helicobacter (HB) infection, comprising determining immunoglobulin G2 (IgG2) anti-H. pylori antibody level in a saliva sample, and predicting likelihood of successful eradication of HB infection in a subject to be treated or being treated for the infection involves determining levels of interleukin-4 (IL-4), interferon- gamma (IFN- gamma) and IgG in the subject, is new.

DETAILED DESCRIPTION - Monitoring efficacy of treatment, eradication of HB infection, relapse or reinfection with HB infection and detecting unresponsiveness of a subject to treatment of HB infection, comprises determining immunoglobulin (Ig)G2 anti-H. pylori antibody level in a saliva sample, and comparing the IgG2 anti-H. pylori antibody level with a predetermined control IgG2 anti-H. pylori antibody level, where a reduction in the level of IgG2 anti-H. pylori antibody in

the saliva sample compared to the control, indicates eradication of HB, efficacious treatment of HB, an increase in the antibody level indicates relapse or reinfection with HB, and lack of change in the level of IgG2 anti-H. pylori antibody in the saliva sample compared to the control indicates lack of response to treatment. Predicting the likelihood of successful eradication of HB infection in a subject to be treated or being treated for the infection, comprises determining IL-4, IFN-gamma , IgG levels and/or their combinations in a sample from the subject, and comparing the levels with a predetermined control or standard level. A level of IL-4 in the sample from the subject above the control or standard IL-4 level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard IL-4 level is predictive of the likelihood of eradication failure, and a level of IFN- gamma or IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IFN- gamma or IgG above the control or standard level is predictive of the likelihood of eradication failure.

An INDEPENDENT CLAIM is also included for a kit for monitoring treatment of HB infection, including HB antigen and reagent for determining IgG2 subclass antibody.

USE - For monitoring progress of treatment and/or eradication of H. pylori infection in a subject undergoing treatment, and for predicting the likelihood of successful eradication of HB infection in a subject to be treated or being treated. The method is also useful for monitoring relapse or reinfection with HB and detecting unresponsiveness of a subject to treatment of HB infection.

ABSTRACTED-PUB-NO: WO 200203065A
EQUIVALENT-ABSTRACTS:

CHOSEN-DRAWING: Dwg.0/7

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CLAIM PTO/ TJ

1. A method of monitoring eradication of Helicobacter infection in a subject treated for the infection, including:

   i) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

   ii) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein a reduction in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates eradication of Helicobacter.

2. A method of monitoring efficacy of treatment of Helicobacter infection in a subject treated for the infection, including:

   i) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

   ii) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein a reduction in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates efficacious treatment of Helicobacter.

3. A method of monitoring relapse or reinfection with Helicobacter in a subject treated for infection with Helicobacter, including:

i) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

ii) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein an increase in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates relapse or reinfection with Helicobacter.

4. A method of detecting unresponsiveness of a subject to treatment of Helicobacter infection, including:

(i) determination of IgG2 anti-*H. pylori* antibody level in a saliva sample;

(ii) comparison of the IgG2 anti-*H. pylori* antibody level with a predetermined control IgG2 anti-*H. pylori* antibody level, wherein lack of change in the level of IgG2 anti-*H. pylori* antibody in the saliva sample compared to the control indicates lack of response to treatment.

5. A method according to any one of claims 1 to 4, wherein the IgG2 anti-*H. pylori* antibody is detected by an immunoassay.

6. A method according to claim 5, wherein the assay is ELISA.

-- 7. (Amended) A method according to any one of claims 1 to 4, wherein the control levels of IgG2 anti-*H. pylori* antibody is established in samples of saliva obtained from subjects not infected by *H. pylori*.

8. (Amended) A method according to any one of claims 1 to 4, wherein the control levels of IgG2 anti-*H. pylori* antibody are determined in subject's own saliva sample.

9. A kit for monitoring treatment of Helicobacter infection, including,

(i) Helicobacter antigen

(ii) reagent for determining IgG2 subclass antibody.

10. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination of IL-4 level in a sample from the subject;

(ii) comparison of the IL-4 level with a predetermined control or standard IL-4 level,

(iii) wherein a level of IL-4 in the sample from the subject above the control or standard IL-4 level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard IL-4 level is predictive of the likelihood of eradication failure.

11. A method according to claim 10 wherein the sample is a blood sample.

12. (Amended) A method according to claim 10, wherein the IL-4 is detected by an immunoassay.

13. A method according to claim 12, wherein the assay is ELISA.

14. (Amended) A method according to any one of claims 10 to 13 and 25, wherein the control or standard level of IL-4 is established from analysis of samples obtained from subjects not infected by *H. pylori* and/or subjects having successfully eradicated *H. pylori* and/or subjects infected by *H. pylori*.

15. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination of interferon-γ (INF-γ) level in a sample from the subject;

(ii) comparison of the INF-γ level with a predetermined control or standard INF-γ level,

(iii) wherein a level of INF-γ in the sample from the subject below the control or standard INF-γ level is predictive of the likelihood of successful eradication and a level of IFN- γ above the control or standard level is predictive of the likelihood of eradication failure.

16. A method according to claim 15 wherein the sample is a blood sample.

17. A method according to claim 15 or claim 16, wherein the IFN- γ level is detected by an immunoassay.

18. A method according to claim 17, wherein the assay is ELISA.

19. (Amended) A method according to claims 15 to 16, wherein the control or standard level of IFN-γ is established from analysis of samples obtained from subjects not infected by *H. pylori* and/or subjects having successfully eradicated *H. pylori* and/or subjects infected by *H. pylori*.–

20. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination of immunoglobulin G (IgG) level in a sample from the subject;

(ii) comparison of the IgG level with a predetermined control or standard IgG level,

(iii) wherein a level of IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

21. A method according to claim 20 wherein the sample is a serum sample.

22. A method according to claim 20 wherein the sample is a saliva sample.

23. A method according to any one of claims 20 to 22, wherein the control or standard level of IgG is established from analysis of samples obtained from subjects not infected by *H. pylori* and/or subjects having successfully eradicated *H. pylori* and/or subjects infected by *H. pylori*.

24. A method of predicting the likelihood of successful eradication of Helicobacter infection in a subject to be treated or being treated for the infection, including:

(i) determination a combination of IL-4 and/or INF-γ and/or IgG levels in a sample from the subject;

(ii) comparison of the IL-4 and/or INF-γ and/or IgG levels with a predetermined control or standard IL-4 and/or INF-γ and/or IgG level respectively,

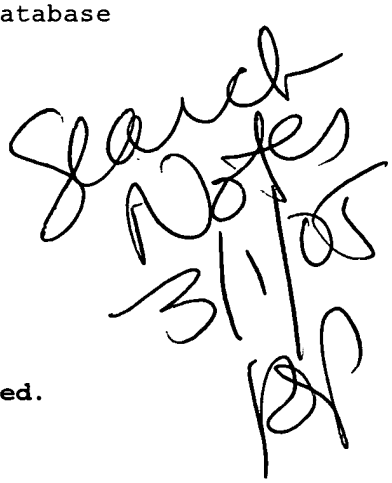
wherein a level of IL-4 in the sample from the subject above the control or standard level is predictive of the likelihood of successful eradication and a level of IL-4 below the control or standard level is predictive of the likelihood of eradication failure, and

wherein a level of INF-γ in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IFN-γ above the control or standard level is predictive of the likelihood of eradication failure, and

wherein a level of IgG in the sample from the subject below the control or standard level is predictive of the likelihood of successful eradication and a level of IgG above the control or standard level is predictive of the likelihood of eradication failure.

25. (New) A method according to claim 11, wherein the IL-4 is detected by an immunoassay.

service. Enter a BEGIN command plus a file number to search a database
(e.g., B1 for ERIC).
?b 155

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File 155:MEDLINE(R) 1951-2005/Feb W3
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*File 155: Medline has been reloaded; acession numbers have changed.
Please see HELP NEWS 154.

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           20687  PYLORI
           20388  HELICOBACTER
          447840  PY=1998
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          422732  PY=1996
           20687  PYLORI
           20388  HELICOBACTER
      S2       2  AU=MOHAMMADI ? AND PY=1996 AND PYLORI AND HELICOBACTER
?s au=strober ? and py=1997 and pylori and helicobacter
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          432935  PY=1997
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           16145  GASTRIN?
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      S4       1  AU=JASSEL ? AND PY=1999 AND GASTRIN? AND PYLORI
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S4          1   AU=JASSEL ? AND PY=1999 AND GASTRIN? AND PYLORI
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Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Screening for **Helicobacter pylori** in dyspeptic patients may improve selectivity for gastroscopy. Rapid serological tests based on ELISA technique are cheap, readily available and simple to use in the clinical setting. However local evaluation is essential in order to validate these techniques. Fifty-six dyspeptic patients (aged less than 45 yr) had a rapid serological test (Helisal) performed prior to gastroscopy. At gastroscopy H. **pylori** status was assessed using culture and histology. The Helisal sensitivity was 80 per cent, specificity 82 per cent. Screening patients with the Helisal test would have missed 6 patients with peptic ulcer disease and 2 with oesophagitis. The Helisal test did not perform satisfactorily as a screening test in selection of patients for gastroscopy.

Tags: Female; Male

Descriptors: *Dyspepsia--microbiology--MI; * **Helicobacter** Infections --diagnosis--DI; * **Helicobacter pylori** --isolation and purification--IP; Adolescent; Adult; Gastroscopy; Humans; Middle Aged; Sensitivity and Specificity; Serologic Tests

Record Date Created: 19981104
Record Date Completed: 19981104

7/9/4

DIALOG(R)File 155:MEDLINE(R)

12276891 PMID: 9588834

Oral tolerance and its modulation by anti-cytokines.

Marth T; **Strober W**

Internal Medicine II, University of the Saarland, Homburg/Saar, Germany.

Research in immunology (FRANCE) Oct-Dec **1997** , 148 (8-9) p554-61, ISSN 0923-2494 Journal Code: 8907467

Publishing Model Print
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
(64 Refs.)

Descriptors: *Cytokines--immunology--IM; *Immune Tolerance--immunology --IM; Animals; Neutralization Tests

CAS Registry No.: 0 (Cytokines)

Record Date Created: 19980625
Record Date Completed: 19980625

7/9/5

DIALOG(R)File 155:MEDLINE(R)

12276884 PMID: 9588827

Peyer's patch dendritic cells and the induction of mucosal immune responses.

Kelsall B L; **Strober W**

Mucosal Immunity Section, Laboratory for Clinical Investigation, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20892-1890, USA.

Research in immunology (FRANCE) Oct-Dec **1997** , 148 (8-9) p490-8, ISSN 0923-2494 Journal Code: 8907467

Publishing Model Print
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

(65 Refs.)
Descriptors: *Dendritic Cells--immunology--IM; *Immunity, Mucosal --immunology--IM; *Peyer's Patches--immunology--IM; Animals; Humans; Immunophenotyping
Record Date Created: 19980625
Record Date Completed: 19980625

7/9/6
DIALOG(R)File 155:MEDLINE(R)

12276883 PMID: 9588826
Tolerance and immunity in the mucosal immune system. Introduction.
Strober W ; Coffman R L
National Institutes of Health, Mucosal Immunity Section, Laboratory of Clinical Investigation, Bethesda, MD 20892-1890, USA.
Research in immunology (FRANCE) Oct-Dec **1997** , 148 (8-9) p489-599, ISSN 0923-2494 Journal Code: 8907467
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Descriptors: *Immune Tolerance--immunology--IM; *Immunity, Mucosal --immunology--IM; Adjuvants, Immunologic
CAS Registry No.: 0 (Adjuvants, Immunologic)
Record Date Created: 19980625
Record Date Completed: 19980625

7/9/7
DIALOG(R)File 155:MEDLINE(R)

12242608 PMID: 9550888
Does adjunctive fluoxetine influence the post-hospital course of restrictor-type anorexia nervosa? A 24-month prospective, longitudinal followup and comparison with historical controls.
Strober M ; Freeman R; DeAntonio M; Lampert C; Diamond J
Department of Psychiatry, University of California at Los Angeles, USA.
Psychopharmacology bulletin (UNITED STATES) **1997** , 33 (3) p425-31, ISSN 0048-5764 Journal Code: 0101123
Publishing Model Print
Document type: Clinical Trial; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
A 24-month naturalistic, prospective longitudinal followup study was conducted on 33 patients with anorexia nervosa who had participated in an intensive, multidisciplinary inpatient treatment program and were receiving fluoxetine as part of their continuing treatment regimen. Data on course, outcome, and treatment exposure in this cohort were obtained using standardized, comprehensive interviews administered at 6-month intervals after hospital discharge. Longitudinal course data for these patients were compared with data for matched historical controls who had received identical inpatient and followup treatment but without adjunctive fluoxetine. Analyses failed to show that fluoxetine had a significant effect on the cumulative probability of remaining at target weight during the followup period, the risk of sustained weight loss, or other clinical measures of outcome. Thus, adjunctive treatment with fluoxetine may not have additive long-term therapeutic benefit when measured against the effects of sustained and intensive followup treatment.
Tags: Comparative Study; Female
Descriptors: *Anorexia Nervosa--drug therapy--DT; *Antidepressive Agents,

S5 2 AU=LEHMANN ? AND PY=1996 AND PYLORI AND HELICOBACTER?
S6 22 AU=STROBER ? AND PY=1997
?s s1 or s2 or s4 or s5 or s6
4 S1
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?t s7/9/all

7/9/1
DIALOG(R)File 155:MEDLINE(R)

12703354 PMID: 10627886
The rise in circulating gastrin **with age is due to increases in gastric autoimmunity and Helicobacter** pylori **infection.**
Jassel S V ; Ardill J E; Fillmore D; Bamford K B; O'Connor F A; Buchanan K D
Altnagelvin Hospital, Londonderry, Northern Ireland.
QJM - monthly journal of the Association of Physicians (ENGLAND) Jul **1999** , 92 (7) p373-7, ISSN 1460-2725 Journal Code: 9438285
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
To assess the effect of increasing age on circulating **gastrin** , we surveyed serum **gastrin** , Helicobactor **pylori** seroantibody status and gastric autoimmunity in 366 hospitalized patients aged 15-90 years. Data were subjected to multivariate analysis, using logarithmic transformation to normalize the distribution of **gastrin** concentrations (presented as geometric means and 95% CIs). The frequency of H. **pylori** -positive antibody status increased with age from 28% in the second decade to > 70% beyond the fourth decade. Fasting **gastrin** concentrations rose significantly from 44 ng/l (41-48) in the second decade to 95 ng/l (67-131) by the eighth decade (p = 0.001) in the total group. Twenty-seven patients (6.8% of the total) tested positive for gastric auto-antibodies: 2% of patients in the second decade, rising to 15.9% in the eighth decade. These patients formed a distinct group with respect to circulating **gastrin** concentrations. Excluding these 27, fasting **gastrin** concentrations still rose significantly, from 44 ng/l (41-48) in the second decade, to 67 ng/l (50-89) in the eighth decade (p = 0.003) in the remaining 341 patients. Fasting **gastrin** concentrations were significantly higher in patients who were H. **pylori** -seropositive (59 ng/l, 54-64 vs. sero-negative 41 ng/l, 37-46) (p = 0.002), and there was no increase in circulating **gastrin** concentrations with increasing age in either the H. **pylori** -positive or the H. **pylori** -negative group. The increase in circulating fasting **gastrin** observed with increasing age is due to an increased incidence of gastric antibodies associated with auto-immune atrophic gastritis, and an increased incidence of H. **pylori** infection.
Descriptors: *Aging--blood--BL; *Antibodies, Bacterial--blood--BL; * **Gastrins** --blood--BL; *Helicobacter Infections--blood--BL; *Helicobacter **pylori** --immunology--IM; Adolescent; Adult; Aged; Aged, 80 and over; Aging --immunology--IM; Anemia, Pernicious--blood--BL; Anemia, Pernicious --immunology--IM; Autoantibodies--analysis--AN; Helicobacter Infections --immunology--IM; Humans; Intrinsic Factor--immunology--IM; Middle Aged; Multivariate Analysis; Parietal Cells, Gastric--immunology--IM
CAS Registry No.: 0 (Antibodies, Bacterial); 0 (Autoantibodies); 0 (Gastrins); 9008-12-2 (Intrinsic Factor)
Record Date Created: 20000201
Record Date Completed: 20000201

7/9/2

DIALOG(R)File 155:MEDLINE(R)

12555969 PMID: 9872618

A prospective study of the management of the young Helicobacter pylori **negative dyspeptic patient--can gastroscopies be saved in clinical practice?**

Heaney A; Collins J S; Tham T C; Watson P R; McFarland J R; **Bamford K B**
Day Procedure Unit, Royal Victoria Hospital, Belfast, Northern Ireland.

European journal of gastroenterology & hepatology (ENGLAND) Nov **1998** , 10 (11) p953-6, ISSN 0954-691X Journal Code: 9000874
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

BACKGROUND: **Helicobacter pylori** status has been suggested as a means of selecting young dyspeptic patients for gastroscopy as patients who are H. **pylori** negative and do not exhibit alarm symptoms or ingest non-steroidal anti-inflammatory medication have a low risk of serious organic disease. AIM: To determine if young patients with ulcer-like dyspepsia and found to be H. **pylori** negative on non-invasive testing could be reassured by this knowledge and not proceed to gastroscopy. PATIENTS: One hundred and sixty-one consecutive attendees aged 45 years or less with a presenting complaint of epigastric pain or discomfort were prospectively recruited from open access gastroscopy referrals and gastroenterology clinics. METHODS: Patients who were H. **pylori** negative on 13-carbon urea breath test were reassured of the likelihood of a normal gastroscopy, given lifestyle advice and also advised to take symptomatic therapy as required. Patients were reviewed at 6 weeks, 3 months and 6 months when symptoms and quality of life were reassessed. Patients proceeded to gastroscopy if at any review their dyspepsia score stayed the same or worsened. RESULTS: Fifty-five H. **pylori** negative patients were recruited (30 male, mean age 31 years), two patients did not attend subsequent review. Thirty-two (58%) came to gastroscopy. Endoscopic diagnoses included 25 which were normal, three with gastro-oesophageal reflux disease, three with peptic ulcer disease and one with gastric erosions. Dyspepsia and quality of life scores showed significant improvement over 6 months. CONCLUSIONS: This management strategy resulted in a 42% reduction in gastroscopies in H. **pylori** negative patients. Whilst the majority of patients endoscoped had normal findings, seven patients (22%) had pathology. Overall there were significant improvements in dyspepsia and quality of life at 6 month follow-up.

Tags: Female; Male
Descriptors: *Dyspepsia--therapy--TH; *Gastroscopy--utilization--UT; *Helicobacter Infections--diagnosis--DI; Adult; Dyspepsia--microbiology --MI; **Helicobacter pylori** --isolation and purification--IP; Humans; Middle Aged; Prognosis; Prospective Studies; Quality of Life
Record Date Created: 19990225
Record Date Completed: 19990225

7/9/3

DIALOG(R)File 155:MEDLINE(R)

12467937 PMID: 9780563

Rapid serological diagnosis of Helicobacter pylori **: a need for caution and re-evaluation.**

Heaney A; Collins J S; Watson R G; McFarland R J; **Bamford K B**
Day Procedure Unit, Royal Victoria Hospital, Belfast, Northern Ireland.

Irish journal of medical science (IRELAND) Jul-Sep **1998** , 167 (3) p152-4, ISSN 0021-1265 Journal Code: 7806864
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH

for whom follow-up data were available had remote seizures. Infants with grade 4 IVH had significantly more acute seizures than infants with grades 1 and 2. In this cohort, only infants with grades 3 and 4 IVH developed remote seizures. Furthermore, among infants with grade 4 IVH acute seizures were a significant risk factor for development of remote seizures. The use of long-term antiepileptic drug therapy in neonates with a history of acute seizures is not established. These results suggest that antiepileptic drug therapy beyond the neonatal period should be reserved for infants with grade 4 IVH with history of acute seizures.

Descriptors: *Cerebral Hemorrhage--complications--CO; *Infant, Premature, Diseases; *Seizures--etiology--ET; Acute Disease; Anticonvulsants --therapeutic use--TU; Cerebral Hemorrhage--etiology--ET; Cohort Studies; Gestational Age; Humans; Infant; Infant, Newborn; Seizures--drug therapy --DT; Severity of Illness Index

CAS Registry No.: 0 (Anticonvulsants)

Record Date Created: 19980105

Record Date Completed: 19980105

7/9/11

12092592 PMID: 9392531

Long-term results of total lymphoid irradiation in the treatment of cardiac allograft rejection.

Wolden S L; Tate D J; Hunt S A; **Strober S** ; Hoppe R T

Department of Radiation Oncology, Stanford University, CA, USA.

International journal of radiation oncology, biology, physics (UNITED STATES) Dec 1 **1997** , 39 (5) p953-60, ISSN 0360-3016 Journal Code: 7603616

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; AIDS/HIV

PURPOSE: To evaluate the short and long-term effects of total lymphoid irradiation (TLI) in the treatment of cardiac transplant rejection. METHODS AND MATERIALS: Between 1986 and 1995, 48 courses of TLI were delivered to 47 cardiac transplant patients. In 37 patients, TLI was administered for intractable allograft rejection despite conventional therapy while 10 patients received TLI prophylactically. The prescribed radiation dose was 8 Gy in 0.8 Gy fractions twice weekly to mantle and inverted-Y plus spleen fields. Postirradiation follow-up ranged from 6 months to 9.1 years, with a mean of 3.1 years. RESULTS: The actual mean dose was 7.3 Gy delivered over a mean of 39 days. Fifty-six percent of patients required treatment delay or abbreviation because of thrombocytopenia, leukopenia, infection, or unrelated problems. In patients treated for intractable rejection, rejection rates dropped from 0.46 to 0.14 and to 0.06 episodes/patient/month before, during, and after TLI ($p < 0.0001$). Rejection rates continued to drop throughout follow-up. Prednisone requirements decreased from 0.41 mg/kg before treatment to 0.21 mg/kg afterward ($p < 0.0001$). The ratio of helper to cytotoxic-suppressor T-cells decreased during TLI from 1.33 to 0.89, and remained low at 0.44, 2-4 months after treatment. Infection rates were not increased and two patients developed malignancy. Rejection rates were high during prophylactic treatment and this protocol was abandoned. Three-year actuarial survival after irradiation was 60% for patients with intractable rejection and 70% for the prophylactic cohort. CONCLUSION: TLI is an effective treatment for control of intractable cardiac rejection. Episodes of rejection and steroid dosage requirements are decreased for up to 9.1 years. A possible mechanism of action is long term alteration in T-lymphocyte subsets. Patients experience transient bone marrow suppression but no increase in infection or bleeding. Long-term complications of TLI are not appreciably different than conventional immunosuppression.

Tags: Female; Male

Descriptors: *Graft Rejection--radiotherapy--RT; *Heart Transplantation; *Lymphatic Irradiation; Adolescent; Adult; Anti-Inflammatory Agents, Non-Steroidal--therapeutic use--TU; CD4-CD8 Ratio; Cause of Death; Child; Child, Preschool; Follow-Up Studies; Graft Rejection--mortality--MO; Graft Rejection--prevention and control--PC; Humans; Infant; Infection--etiology --ET; Lymphatic Irradiation--adverse effects--AE; Middle Aged; Prednisone --therapeutic use--TU; Radiotherapy Dosage; Thrombocytopenia--etiology--ET

CAS Registry No.: 0 (Anti-Inflammatory Agents, Non-Steroidal); 53-03-2 (Prednisone)

Record Date Created: 19971211

Record Date Completed: 19971211

7/9/12

DIALOG(R)File 155:MEDLINE(R)

12063141 PMID: 9356884

The long-term course of severe anorexia nervosa in adolescents: survival analysis of recovery, relapse, and outcome predictors over 10-15 years in a prospective study.

Strober M ; Freeman R; Morrell W

Neuropsychiatric Institute and Hospital, School of Medicine, University of California at Los Angeles, USA.

International journal of eating disorders (UNITED STATES) Dec **1997** , 22 (4) p339-60, ISSN 0276-3478 Journal Code: 8111226

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

OBJECTIVE: To assess the long-term course of recovery and relapse and predictors of outcome in anorexia nervosa. METHOD: A naturalistic, longitudinal prospective design was used to assess recovery and relapse in patients ascertained through a university-based specialty treatment program. Patients were assessed semiannually for 5 years and annually thereafter over 10-15 years from the time of their index admission. Recovery was defined in terms of varying levels of symptom remission maintained for no fewer than 8 consecutive weeks. RESULTS: Nearly 30% of patients had relapses following hospital discharge, prior to clinical recovery. However, most patients were weight recovered and menstruating regularly by the end of follow-up, with nearly 76% of the cohort meeting criteria for full recovery. Relapse after recovery was relatively uncommon. Of note, time to recovery was protracted, ranging from 57-79 months depending on definition of recovery. Among restrictors at intake, nearly 30% developed binge eating, occurring within 5 years of intake. A variety of predictors of chronic outcome and binge eating were identified. There were no deaths in the cohort. CONCLUSION: The course of anorexia nervosa is protracted. Predictors of outcome are surprisingly few, but those identified are in keeping with previous accounts. The intensive treatment received by these patients may account for the lower levels of morbidity and mortality when considered in relation to other reports in the follow-up literature.

Tags: Female

Descriptors: *Anorexia Nervosa--epidemiology--EP; Adaptation, Psychological; Adolescent; Adult; Anorexia Nervosa--psychology--PX; Anorexia Nervosa--therapy--TH; Body Mass Index; California--epidemiology --EP; Child; Cohort Studies; Follow-Up Studies; Humans; Patient Admission --statistics and numerical data--SN; Prospective Studies; Recurrence; Survival Analysis; Treatment Outcome

Record Date Created: 19971202

Record Date Completed: 19971202

7/9/13

DIALOG(R)File 155:MEDLINE(R)

12028302 PMID: 9317162

TGF-beta production regulates the development of the 2,4,6-trinitrophenol-conjugated keyhole limpet hemocyanin-induced colonic inflammation in IL-2-deficient mice.

Ludviksson B R; Ehrhardt R O; **Strober W**

Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA.

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Oct 1 **1997** , 159 (7) p3622-8, ISSN 0022-1767 Journal Code: 2985117R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS; AIDS/HIV

A severe, Th1-mediated experimental colitis with similarities to inflammatory bowel disease in humans can be induced by a single injection of 2,4,6-trinitrophenol (TNP)-substituted protein plus adjuvant in IL-2-/- mice. To determine the early events involved in the pathogenesis of IL-2-/-colitis, we compared the function of lamina propria (LP) T cells from IL-2-/- and IL-2+/+ mice subjected to disease-inducing (TNP-conjugated keyhole limpet hemocyanin [TNP-KLH]) and disease-inhibiting (anti-CD3) immunization protocols. We show that LP T cells in TNP-KLH-immunized IL-2-/- mice fail to produce TGF-beta early (day 2), whereas LP T cells in TNP-KLH-immunized IL-2+/+ mice exhibit an approximately eightfold rise in TGF-beta secretion. The critical importance of local TGF-beta production was further substantiated by the following findings. 1) LP T cells from TNP-KLH-immunized IL-2-/- mice administered anti-CD3 (i.p.) exhibit a significant rise in TGF-beta, production but fail to produce IFN-gamma, and such mice do not develop colitis. 2) TNP-KLH-immunized IL-2-/- mice administered anti-CD3 and coadministered anti-TGF-beta mAb again give rise to IFN-gamma-producing LP cells, and such mice develop colitis. 3) TNP-KLH-immunized IL-2+/+ mice administered anti-TGF-beta mAb exhibit pockets of mononuclear cell infiltrates in the LP. These results indicate that the disposition of IL-2-/- mice to develop chronic colonic inflammation is due to a Th1 cell response in the LP that is not appropriately counter-regulated by the production of the suppressor cytokine, TGF-beta.

Tags: Female; Male

Descriptors: *Colitis--etiology--ET; *Hemocyanin--immunology--IM; *Interleukin-2--deficiency--DF; *Picrates--immunology--IM; *Transforming Growth Factor beta--biosynthesis--BI; Animals; Antibodies, Monoclonal --administration and dosage--AD; Antigens, CD3--immunology--IM; Colitis --immunology--IM; Colitis--prevention and control--PC; Hemocyanin --metabolism--ME; Interleukin-2--genetics--GE; Interleukin-4--biosynthesis --BI; Interleukin-4--deficiency--DF; Mice; Mice, Knockout; Th1 Cells --immunology--IM; Transforming Growth Factor beta --antagonists and inhibitors--AI; Transforming Growth Factor beta--deficiency--DF

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Antigens, CD3); 0 (Interleukin-2); 0 (Picrates); 0 (Transforming Growth Factor beta); 0 (trinitrophenyl keyhole limpet hemocyanin); 207137-56-2 (Interleukin-4); 88-89-1 (picric acid); 9013-72-3 (Hemocyanin)

Record Date Created: 19971021

Record Date Completed: 19971021

7/9/14

12000310 PMID: 9285262

Psychiatric disorders in women with bulimia nervosa and their first-degree relatives: effects of comorbid substance dependence.

Lilenfeld L R; Kaye W H; Greeno C G; Merikangas K R; Plotnicov K; Pollice C; Rao R; **Strober M** ; Bulik C M; Nagy L

Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine, PA 15213, USA.

International journal of eating disorders (UNITED STATES) Nov **1997** , 22 (3) p253-64, ISSN 0276-3478 Journal Code: 8111226

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

OBJECTIVE: Women with bulimia nervosa (BN) and comorbid substance dependence often display impulsive behaviors. We assessed Axis I and II psychiatric diagnoses in their first-degree relatives in order to understand the etiological factors that may contribute to this subtype of BN. METHOD: We used contemporary family-epidemiological methodology to compare the lifetime prevalence of psychiatric disorders among 47 women with BN and 44 non-eating-disordered community control women, and their first-degree relatives (177 and 190, respectively). BN probands were stratified by the presence (n = 20) or absence (n = 27) of a lifetime history of alcohol and/or drug dependence. RESULTS: Social phobia, conduct disorder, and clusters B and C personality disorders were significantly more prevalent among BN probands with substance dependence than among BN probands without substance dependence or control women probands. Substance use disorders, social phobia, panic disorder, and cluster B personality disorders were significantly more prevalent among the relatives of BN probands with substance dependence than the relatives of the other two groups. DISCUSSION: Women with BN and substance dependence have problems with social anxiety, antisocial behavior, and a variety of personality disturbances, and come from families where there are problems with substance use disorders, anxiety, impulsivity, and affective instability. These data raise the possibility that a familial vulnerability for impulsivity and affective instability may contribute to the development of substance dependence in a subgroup of women with BN.

Tags: Female

Descriptors: *Bulimia--epidemiology--EP; *Family Health; *Substance-Related Disorders--epidemiology--EP; Adult; Anxiety Disorders--epidemiology--EP; Case-Control Studies; Comorbidity; Humans; Mood Disorders--epidemiology--EP ; Pennsylvania--epidemiology--EP; Personality Disorders--epidemiology--EP; Prevalence

Record Date Created: 19971023

Record Date Completed: 19971023

7/9/15

DIALOG(R)File 155:MEDLINE(R)

11971423 PMID: 9255619

From stem cells to lymphocytes: biology and transplantation.

Aguila H L; Akashi K; Domen J; Gandy K L; Lagasse E; Mebius R E; Morrison S J; Shizuru J; **Strober S** ; Uchida N; Wright D E; Weissman I L

Department of Pathology, Stanford University School of Medicine, California 94305, USA.

Immunological reviews (DENMARK) Jun **1997** , 157 p13-40, ISSN 0105-2896 Journal Code: 7702118

Publishing Model Print

Document type: Historical Article; Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

We review the development of the hematopoietic system, focusing on the transition from hematopoietic stem cells (HSCs) to T cells. This includes the isolation of HSCs, and recent progress in understanding their ontogeny, homing properties, and differentiation. HSC transplantation is reviewed, including the kinetics of reconstitution, engraftment across

histocompatibility barriers, the facilitation of allogeneic engraftment, and the mechanisms of graft rejection. We describe progress in understanding T-cell development in the bone marrow and thymus as well as the establishment of lymph nodes. Finally, the role of bcl-2 in regulating homeostasis in the hematopoietic system is discussed. (282 Refs.)

Descriptors: *Hematopoietic Stem Cell Transplantation--history--HI; *Hematopoietic Stem Cells--immunology--IM; *Transplantation Immunology; Animals; Hematopoietic Stem Cell Transplantation--methods--MT; History, 20th Century; Humans

Record Date Created: 19970916

Record Date Completed: 19970916

7/9/16

11963574 PMID: 9247586

Predominant pathogenic role of tumor necrosis factor in experimental colitis in mice.

Neurath M F; Fuss I; Pasparakis M; Alexopoulou L; Haralambous S; Meyer zum Buschenfelde K H; **Strober W** ; Kollias G

Laboratory of Immunology, I. Medical Clinic, University of Mainz, Germany.

European journal of immunology (GERMANY) Jul **1997** , 27 (7) p1743-50 , ISSN 0014-2980 Journal Code: 1273201

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Antibodies to tumor necrosis factor (TNF)-alpha have been recently proposed as effective treatment for patients with Crohn's disease. Here, we analyze the functional role of TNF-alpha in a mouse model of chronic intestinal inflammation induced by the hapten reagent 2,4,6,-trinitrobenzene sulfonic acid (TNBS) that mimics some characteristics of Crohn's disease in humans. Macrophage-enriched lamina propria (LP) mononuclear cells from mice with TNBS-induced colitis produced 10-30-fold higher levels of TNF-alpha mRNA and protein than cells from control mice. When mice with chronic colitis were treated by intraperitoneal injection of antibodies to TNF-alpha, an improvement of both the clinical and histopathologic signs of disease was found. Isolated macrophage-enriched LP cells from anti-TNF-alpha-treated mice produced strikingly less pro-inflammatory cytokines such as interleukin (IL)-1 and IL-6 in cell culture. The predominant role of TNF-alpha in the mouse TNBS-induced colitis model was further underlined by the finding that striking colonic inflammation and lethal pancolitis was induced in TNF-alpha-transgenic mice upon TNBS treatment. Conversely, no significant TNBS-induced colitis could be induced in mice in which the TNF-alpha gene had been inactivated by homologous recombination. Complementation of TNF-alpha function in TNF-/- mice by the expression of a mouse TNF-alpha transgene was sufficient to reverse this effect. Taken together, the data provide direct evidence for a predominant role of TNF-alpha in a mouse model of chronic intestinal inflammation and encourage further clinical trials with antibodies to TNF-alpha for the treatment of patients with Crohn's disease.

Tags: Female; Research Support, Non-U.S. Gov't

Descriptors: *Colitis--etiology--ET; *Colitis--immunology--IM; *Tumor Necrosis Factor-alpha--physiology--PH; Animals; Antibodies--therapeutic use --TU; Chronic Disease; Colitis--mortality--MO; Colitis--pathology--PA; Disease Models, Animal; Inflammatory Bowel Diseases--etiology--ET; Inflammatory Bowel Diseases--immunology--IM; Inflammatory Bowel Diseases --pathology--PA; Mice; Mice, Inbred Strains; Mice, Knockout; Mice, Transgenic; Trinitrobenzenesulfonic Acid--toxicity--TO; Tumor Necrosis Factor-alpha--genetics--GE; Tumor Necrosis Factor-alpha--immunology--IM

CAS Registry No.: 0 (Antibodies); 0 (Tumor Necrosis Factor-alpha);

2508-19-2 (Trinitrobenzenesulfonic Acid)
Record Date Created: 19970825
Record Date Completed: 19970825

7/9/17
DIALOG(R)File 155:MEDLINE(R)

11963451 PMID: 9247462
Defects of monocyte interleukin 12 production and humoral immunity in Whipple's disease.
Marth T; Neurath M; Cuccherini B A; **Strober W**
Mucosal Immunity Section, National Institutes of Health, Bethesda, Maryland, USA. Intmar@med-rz.uni-sb.de
Gastroenterology (UNITED STATES) Aug **1997** , 113 (2) p442-8, ISSN 0016-5085 Journal Code: 0374630
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS
BACKGROUND & AIMS: Whipple's disease (WD) is a systemic infection in which the causative bacteria typically accumulate within macrophages. The aim of this study was to test whether this macrophage dysfunction is the cause or result of previously shown T-cell defects. METHODS: In vitro production of interleukin (IL)-12, IL-10, tumor necrosis factor alpha, interferon gamma (IFN-gamma), and transforming growth factor beta (TGF-beta) from purified monocytes and peripheral blood mononuclear cells, cytokine expression on duodenal biopsy specimens, and serum cytokine and immunoglobulin (Ig) levels were tested in 9 patients with WD. RESULTS: Reduced monocyte IL-12 production and decreased IFN-gamma secretion by peripheral blood mononuclear cells in vitro were found, as well as reduced immunohistological staining for IL-12 and IFN-gamma, but no decrease in other cytokines in patients with WD. A similar but less severe defect in 2 relatives with WD argued for a genetic basis of this abnormality. Serum IgG2, an IFN-gamma-dependent Ig subclass, and serum TGF-beta levels were reduced in patients with WD. CONCLUSIONS: The described monocyte defects in WD may result in a secondary reduction of IFN-gamma production and IgG2 serum levels. This provides a rationale for additive immunotherapy in patients with antibiotic-refractory WD.
Tags: Female; Male; Research Support, Non-U.S. Gov't
Descriptors: *Interleukin-12--biosynthesis--BI; *Monocytes--metabolism --ME; *Whipple Disease--immunology--IM; Antibody Formation--immunology--IM; Cells, Cultured; Cryopreservation; Cytokines--biosynthesis--BI; Duodenum --pathology--PA; Humans; Immunoglobulin G--blood--BL; Immunohistochemistry; Interferon Type II--blood--BL; Interleukin-10--biosynthesis--BI; Macrophages--metabolism--ME; Macrophages--pathology--PA; Macrophages --physiology--PH; Middle Aged; Monocytes--pathology--PA; Monocytes --physiology--PH; T-Lymphocytes--metabolism--ME; T-Lymphocytes--pathology --PA; T-Lymphocytes--physiology--PH; Transforming Growth Factor beta --blood--BL; Tumor Necrosis Factor-alpha--biosynthesis--BI; Whipple Disease --metabolism--ME; Whipple Disease--pathology--PA
CAS Registry No.: 0 (Cytokines); 0 (Immunoglobulin G); 0 (Transforming Growth Factor beta); 0 (Tumor Necrosis Factor-alpha); 130068-27-8 (Interleukin-10); 187348-17-0 (Interleukin-12); 82115-62-6 (Interferon Type II)
Record Date Created: 19970826
Record Date Completed: 19970826

7/9/18
DIALOG(R)File 155:MEDLINE(R)

11927552 PMID: 9207483

Granulocyte colony-stimulating factor reduces the capacity of blood mononuclear cells to induce graft-versus-host disease: impact on blood progenitor cell transplantation.

Zeng D; Dejbakhsh-Jones S; **Strober S**

Department of Medicine, Stanford University School of Medicine, CA 94305-5111, USA.

Blood (UNITED STATES) Jul 1 **1997** , 90 (1) p453-63, ISSN 0006-4971 Journal Code: 7603509

Contract/Grant No.: R01 55793; PHS; R01 58250; PHS

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

The feasibility of transplanting peripheral blood mononuclear cells (PBMC) from granulocyte colony-stimulating factor (G-CSF)-treated normal human donors to myeloablated allogeneic hosts has been demonstrated recently. The current work examined the ability of recombinant G-CSF to alter peripheral blood T-cell function and graft-versus-host disease (GVHD) in a murine model of allogeneic G-CSF-mobilized PBMC transplantation. Administration of recombinant G-CSF to C57BL/Ka mice markedly increased the capacity of PBMC to reconstitute lethally irradiated syngeneic hosts. T- and B-lineage lymphocytes were depleted about 10-fold in the bone marrow of the treated mice, and the T-cell yield in the blood was increased about fourfold. The ability of PBMC or purified CD4+ and CD8+ T cells to induce acute lethal GVHD in irradiated BALB/c mice was reduced after the administration of G-CSF. This was associated with decreased secretion of interferon gamma and interleukin-2 (IL-2) and an increased secretion of IL-4. The donor cell inoculum, which was most successful in the rescue of irradiated allogeneic hosts, was the low-density fraction of PBMC from G-CSF-treated mice. These low-density cells were enriched for CD4-CD8-NK1.1+ T cells and secreted about 10-fold more IL-4 than the unfractionated cells from the G-CSF-treated donors.

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Graft vs Host Disease--prevention and control--PC; *Granulocyte Colony-Stimulating Factor--administration and dosage--AD; *Hematopoietic Stem Cell Transplantation--adverse effects--AE; Animals; Cytokines--secretion--SE; Flow Cytometry; Graft vs Host Disease--etiology --ET; Graft vs Host Disease--immunology--IM; Humans; Immunophenotyping; Killer Cells, Natural--drug effects--DE; Mice; Mice, Inbred BALB C; Mice, Inbred C57BL; Recombinant Proteins--administration and dosage--AD; Transplantation, Homologous

CAS Registry No.: 0 (Cytokines); 0 (Recombinant Proteins); 143011-72-7 (Granulocyte Colony-Stimulating Factor)

Record Date Created: 19970722

Record Date Completed: 19970722

7/9/19

DIALOG(R)File 155:MEDLINE(R)

11896618 PMID: 9174613

Regulation of transforming growth factor-beta production by interleukin-12.

Marth T; **Strober W** ; Seder R A; Kelsall B L

Mucosal Immunity Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892-1890, USA.

European journal of immunology (GERMANY) May **1997** , 27 (5) p1213-20 , ISSN 0014-2980 Journal Code: 1273201

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The induction of peripheral tolerance following oral antigen administration in several autoimmune disease and conventional animal models correlates with the production of transforming growth factor-beta (TGF-beta) and T helper type 2 (Th2) cytokines. The factors regulating TGF-beta production and its relation to the Th2 response, however, have not been defined. We demonstrate that the systemic administration of antibodies to interleukin (IL)-12 to ovalbumin (OVA)-T cell receptor (TCR) transgenic mice fed high doses of OVA, followed by systemic OVA challenge, substantially enhances TGF-beta, but not IL-4 production by peripheral T cells. Furthermore, we demonstrate in an in vitro T cell differentiation model that naive (CD4+/Mel-14hi) OVA-TCR-T cells stimulated with OVA-pulsed dendritic cells (DC) produce four- to fivefold higher amounts of TGF-beta when cultured with anti-IL-12 or anti-interferon-gamma (IFN-gamma). In this in vitro system, IL-4 was not required for TGF-beta production by T cells; however, it appeared to enhance levels of TGF-beta by promoting the growth of TGF-beta-producing cells. Our findings demonstrate that IL-12 and IFN-gamma are important negative regulators of TGF-beta production both in vivo and in vitro, and that their modulation may be of benefit for the treatment of autoimmune disorders.

Tags: Female; Research Support, Non-U.S. Gov't

Descriptors: *Interleukin-12--pharmacology--PD; *Transforming Growth Factor beta--biosynthesis--BI; Adjuvants, Immunologic--pharmacology--PD; Animals; Antibodies, Blocking--pharmacology--PD; Antibodies, Monoclonal --pharmacology--PD; Cells, Cultured; Coculture Techniques; Dendritic Cells --metabolism--ME; Interferon Type II--immunology--IM; Interleukin-12 --immunology--IM; Interleukin-4--physiology--PH; Mice; Mice, Inbred BALB C; Mice, Inbred C57BL; Mice, Transgenic; Ovalbumin--genetics--GE; Receptors, Antigen, T-Cell--genetics--GE; T-Lymphocytes--metabolism--ME; Transforming Growth Factor beta--drug effects--DE

CAS Registry No.: 0 (Adjuvants, Immunologic); 0 (Antibodies, Blocking) ; 0 (Antibodies, Monoclonal); 0 (Receptors, Antigen, T-Cell); 0 (Transforming Growth Factor beta); 187348-17-0 (Interleukin-12); 207137-56-2 (Interleukin-4); 82115-62-6 (Interferon Type II); 9006-59-1 (Ovalbumin)

Record Date Created: 19970630

Record Date Completed: 19970630

7/9/20

DIALOG(R)File 155:MEDLINE(R)

11871658 PMID: 9144862

Dendritic cells of the gastrointestinal tract.

Kelsall B L; **Strober W**

Mucosal Immunity Section, National Institutes for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 22908, USA.

Springer seminars in immunopathology (GERMANY) **1997** , 18 (4) p409-20, ISSN 0344-4325 Journal Code: 7910384

Publishing Model Print

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

(56 Refs.)

Descriptors: *Dendritic Cells; *Digestive System--cytology--CY; Animals; Dendritic Cells--cytology--CY; Dendritic Cells--physiology--PH; Digestive System--immunology--IM; Humans; Immunity, Mucosal

Record Date Created: 19970708

Record Date Completed: 19970708

7/9/21

DIALOG(R)File 155:MEDLINE(R)

11838226 PMID: 9097204

Efficacy of methylphenidate in bulimia nervosa comorbid with attention-deficit hyperactivity disorder: a case report.

Schweickert L A; **Strober M** ; Moskowitz A

Neuropsychiatric Institute and Hospital, School of Medicine, University of California at Los Angeles 90024-1759, USA.

International journal of eating disorders (UNITED STATES) Apr **1997** , 21 (3) p299-301, ISSN 0276-3478 Journal Code: 8111226

Publishing Model Print

Document type: Case Reports; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

This case describes the beneficial effect on the binge eating component of bulimia nervosa of methylphenidate, which was prescribed to treat comorbid attention-deficit/hyperactivity disorder. Possible mechanisms of action are discussed.

Tags: Female

Descriptors: *Attention Deficit Disorder with Hyperactivity--drug therapy --DT; *Bulimia--drug therapy--DT; *Central Nervous System Stimulants --therapeutic use--TU; *Methylphenidate--therapeutic use--TU; Adult; Attention Deficit Disorder with Hyperactivity--epidemiology--EP; Bulimia --epidemiology--EP; Comorbidity; Humans

CAS Registry No.: 0 (Central Nervous System Stimulants); 113-45-1 (Methylphenidate)

Record Date Created: 19970613

Record Date Completed: 19970613

7/9/22

DIALOG(R)File 155:MEDLINE(R)

11823892 PMID: 9116306

Granulocyte colony-stimulating factor-induced comobilization of CD4- CD8- T cells and hematopoietic progenitor cells (CD34+) in the blood of normal donors.

Kusnierz-Glaz C R; Still B J; Amano M; Zukor J D; Negrin R S; Blume K G; **Strober S**

Department of Medicine, Stanford University School of Medicine, CA 94305-5111, USA.

Blood (UNITED STATES) Apr 1 **1997** , 89 (7) p2586-95, ISSN 0006-4971 Journal Code: 7603509

Contract/Grant No.: PO1-CA-49605; CA; NCI; R01-CA-55793; CA; NCI

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

The feasibility of transplantation of HLA-matched hematopoietic progenitor cells from the blood of normal donors given granulocyte colony-stimulating factor (G-CSF) has been reported recently. In the current study, the changes in T-cell subsets as well as CD34+ cells were determined in one blood volume leukapheresis products of six normal individuals given G-CSF. Examination of the T-cell subsets in the leukapheresis products showed three different patterns: one in which a discrete population of CD4- CD8- alphabeta T cells was found in addition to the typical CD4+ and CD8+ T cells in the unfractionated as well as in high- and low-density cells; a second in which the discrete population of CD4- CD8- alphabeta T cells was predominant only in the low-density fractions; and a third in which a discrete population of CD4- CD8- T cells was not observed. The median yield of CD4- CD8- T cells was about fourfold to fivefold higher than the calculated number present in one blood volume (5L) from normal individuals. The ratios of CD34+ cells to CD4+ and CD8+ T

cells, and of CD4- CD8- T cells to CD4+ and CD8+ T cells, were highest in the low-density fractions. These fractions suppressed the mixed leukocyte, and may ameliorate graft-versus-host disease as compared with unfractionated cells.

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Bone Marrow--drug effects--DE; *Granulocyte Colony-Stimulating Factor--pharmacology--PD; *Hematopoietic Stem Cells --drug effects--DE; *T-Lymphocyte Subsets--drug effects--DE; Adult; Antigens, CD34--analysis--AN; Antigens, CD4--analysis--AN; Antigens, CD8 --analysis--AN; Blood Cell Count--drug effects--DE; Blood Donors; Bone Marrow Cells; Centrifugation, Density Gradient; Flow Cytometry; Granulocyte Colony-Stimulating Factor--therapeutic use--TU; Humans; Leukapheresis; Light; Lymphocyte Culture Test, Mixed; Scattering, Radiation; T-Lymphocyte Subsets--immunology--IM

CAS Registry No.: 0 (Antigens, CD34); 0 (Antigens, CD4); 0 (Antigens, CD8); 143011-72-7 (Granulocyte Colony-Stimulating Factor)

Record Date Created: 19970422

Record Date Completed: 19970422

7/9/23

DIALOG(R)File 155:MEDLINE(R)

11804305 PMID: 9057354

Reciprocal IFN-gamma and TGF-beta responses regulate the occurrence of mucosal inflammation.

Strober W ; Kelsall B; Fuss I; Marth T; Ludviksson B; Ehrhardt R; Neurath M

Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA. wstrober@atlas.niaid.nih.gov

Immunology today (ENGLAND) Feb **1997** , 18 (2) p61-4, ISSN 0167-5699 Journal Code: 8008346

Publishing Model Print

Document type: Journal Article; Review; Review, Tutorial

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The above new findings concerning the immunological mechanisms governing mucosa, immune responses and oral tolerance in TCR-transgenic mice, as well as those operative in mice with experimental colitis, greatly expand our understanding of the processes that normally control mucosal inflammation and possibly other types of inflammation as well (Fig. 1). They indicate that, in the nondiseased mouse, ingested proteins evoke a Th1-cell (IFN-gamma) response in the mucosal follicles that is quickly counter-regulated by induction of T-cell anergy/deletion, if this Th1-cell response is inhibited (experimentally by anti-IL-12), TGF beta-producing cells appear, and these are capable of active immune suppression. This reciprocal relationship between IFN-gamma production and TGF-beta production is further supported in mouse models of mucosal inflammation. Thus, in the TNBS-colitis model, there is direct stimulation of the immune cells in the lamina propria as a result of diffuse haptenization of mucosal proteins, which leads to a massive Th1-cell response capable of overwhelming any suppressive counter-regulatory mechanisms normally generated in the PPs. This highly polarized Th1-cell response is controlled only by direct abrogation of IL-12 production with exogenous administration of anti-IL-12, or with indirect inhibition of this response via induction of oral tolerance and accompanying production of TGF-beta (Refs 6-8). The data obtained from this model are consistent with those obtained with another model of intestinal inflammation--inflammation in severe combined immunodeficiency (SCID) mice following lymphoid repletion with CD45Rbhi (naive) T cells. In this model, inflammation is again mediated by Th1 cells and is prevented by co-repletion with CD45Rbhi (memory) T cells, which appear to work by secreting TGF-beta (Refs 9, 10). Thus, a common feature of the various experimental models of intestinal inflammation studied to

date is the Yin-Yang relationship of IFN-gamma and TGF-beta, with the former being proinflammatory and the latter anti-inflammatory. Is the IFN-gamma TGF-beta dichotomy that is evident both in the normal state and in models of inflammation simply a reflection of an underlying Th1 Th2 dichotomy? The answer to this important question is not yet known. Thus, while it is clear from the in vitro studies already discussed that IL-12 and/or IFN-gamma inhibit TGF-beta production, the role of IL-4 in this process is more elusive. These in vitro studies indicate that IL-4 is not required for TGF-beta production, a finding that is consistent with studies in which the transfer of CD45Rbhi, T cells from IL-4-/- mice protected SCID mice from colitis induced by CD45Rbhi T cells. However, the addition of IL-4 to in vitro cultures containing anti-IL-12 augmented TGF-beta production, most probably by IL-4 acting as a growth factor for TGF-beta-producing cells rather than as an inducing factor (T. Marth et al., unpublished). Obviously, more work will be necessary to resolve this issue. Finally, it should be noted that the above considerations apply to human inflammatory diseases of the gastrointestinal tract, such as Crohn's disease. Recently, it has been shown that T cells extracted from Crohn's disease tissues manifest skewed Th1-cell responses. The hypothesis can therefore be put forward that this disease results from a dysregulated Th1-cell response to ubiquitous mucosal antigens that is not appropriately controlled by normal counter-regulatory mechanisms. Interventions that artificially bring the excessive Th1-cell response back into balance, such as administration of IL-12 antagonists, should therefore find a central place in the treatment of the disease. (11 Refs.)

Descriptors: *Colitis--immunology--IM; *Interferon Type II--biosynthesis --BI; *Interferon Type II--physiology--PH; *Intestinal Mucosa--immunology --IM; *Intestinal Mucosa--pathology--PA; *Transforming Growth Factor beta --biosynthesis--BI; *Transforming Growth Factor beta--physiology--PH; Animals; Humans

CAS Registry No.: 0 (Transforming Growth Factor beta); 82115-62-6 (Interferon Type II)

Record Date Created: 19970409

Record Date Completed: 19970409

7/9/24

DIALOG(R)File 155:MEDLINE(R)

11783188 PMID: 9029133

Characteristic T helper 2 T cell cytokine abnormalities in autoimmune lymphoproliferative syndrome, a syndrome marked by defective apoptosis and humoral autoimmunity.

Fuss I J; **Strober W** ; Dale J K; Fritz S; Pearlstein G R; Puck J M; Lenardo M J; Straus S E

Mucosal Immunity Section, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA.

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Feb 15 **1997** , 158 (4) p1912-8, ISSN 0022-1767 Journal Code: 2985117R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS; AIDS/HIV

Autoimmune lymphoproliferative syndrome (ALPS) is marked by massive lymphadenopathy, hepatosplenomegaly, autoimmunity and the presence of increased numbers of circulating and tissue TCR-alpha beta, CD4- CD8- T cells. The underlying defect is that of decreased T cell and B cell apoptosis, due in most, but not all, cases to heterozygous mutations of the Fas gene and corresponding defective Fas signaling function. Here we measure in vivo and in vitro cytokine secretion in ALPS to shed light on the relation of apoptosis defects to the development of autoimmunity. In in vivo studies, ALPS patients manifested greatly increased circulating levels of IL-10 (> 100-fold), compared with both healthy individuals and various disease controls; in contrast, their levels of IL-1 beta, IL-4, and

IFN-gamma were normal and their levels of IL-2 and TNF-alpha were marginally increased. In parallel in vitro studies, ALPS patients CD4+ DR+ T cells stimulated either with anti-CD3/CD28 or anti-CD2/CD28 produced increased amounts of IL-4 and IL-5 (10 to 20-fold) and decreased amounts of IFN-gamma (4-fold) as compared with those of control CD4+ DR+ T cells. In contrast, ALPS patients' CD4-/CD8- T cells produced very low amounts of cytokines. Finally, ALPS patients' peripheral monocytes/macrophages produced decreased amounts of IL-12 (30-fold) and increased amounts of IL-10 (5-fold). In conclusion, ALPS is marked by the presence of DR+ T cells that exhibit a skewed Th2 cytokine response upon various forms of stimulation. This cytokine response, in the presence of increased circulating IL-10 levels, is likely to define the cytokine milieu that accounts for the humoral autoimmune features of ALPS and, perhaps, of other humoral autoimmune states.

Descriptors: *Apoptosis--immunology--IM; *Autoantibodies--biosynthesis --BI; *Autoimmune Diseases--metabolism--ME; *Cytokines--biosynthesis--BI; *Lymphoproliferative Disorders--immunology--IM; *Th2 Cells--metabolism--ME ; Adolescent; Adult; Autoimmune Diseases--immunology--IM; B-Lymphocyte Subsets--metabolism--ME; CD4-Positive T-Lymphocytes--immunology--IM; CD4-Positive T-Lymphocytes--secretion--SE; CD8-Positive T-Lymphocytes --secretion--SE; Child; Child, Preschool; Cytokines--blood--BL; HLA-DR Antigens--analysis--AN; Humans; Immunophenotyping; Infant; Interleukin-10 --biosynthesis--BI; Interleukin-10--blood--BL; Lymphokines--secretion--SE; Lymphoproliferative Disorders--metabolism--ME; Macrophages--metabolism--ME; Monocytes--metabolism--ME; Syndrome; T-Lymphocyte Subsets--metabolism--ME

CAS Registry No.: 0 (Autoantibodies); 0 (Cytokines); 0 (HLA-DR Antigens); 0 (Lymphokines); 130068-27-8 (Interleukin-10)

Record Date Created: 19970305

Record Date Completed: 19970305

7/9/25

DIALOG(R)File 155:MEDLINE(R)

11783038 PMID: 9028957

Clincial, immunologic, and genetic features of an autoimmune lymphoproliferative syndrome associated with abnormal lymphocyte apoptosis.

Sneller M C; Wang J; Dale J K; **Strober W** ; Middelton L A; Choi Y; Fleisher T A; Lim M S; Jaffe E S; Puck J M; Lenardo M J; Straus S E

Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

Blood (UNITED STATES) Feb 15 **1997** , 89 (4) p1341-8, ISSN 0006-4971 Journal Code: 7603509

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

Programmed cell death (apoptosis) of activated lymphocytes is critical to immune homeostasis. The cell surface protein Fas (CD95) and its ligand play a pivotal role in regulating lymphocyte apoptosis, and defective expression of either Fas or Fas ligand results in marked over accumulation of mature lymphocytes and autoimmune disease in mice. The results of recent studies suggest that defective lymphocyte apoptosis caused by mutations of the Fas gene can result in a severe autoimmune lymphoproliferative syndrome (ALPS) in humans. To define the clinical, genetic, and immunologic spectrum of ALPS, 9 patients and their families were extensively evaluated with routine clinical studies, lymphocyte phenotyping, genotyping, and in vitro assays for lymphocyte apoptosis. Individual patients were followed up for 3 months to 6 years. ALPS was identified in 9 unrelated children as manifested by moderate to massive splenomegaly and lymphadenopathy, hypergammaglobulinemia, autoimmunity, B-cell lymphocytosis, and the expansion of an unusual population of CD4- CD8- T cells that express the alpha/beta T-cell receptor (TCR). All patients showed defective lymphocyte

apoptosis in vitro. Heterozygous mutations of the Fas gene were detected in 8 patients. One ALPS patient lacked a Fas gene mutation. Healthy relatives with Fas mutations were identified in 7 of 8 ALPS kindreds. These relatives also showed in vitro abnormalities of Fas-mediated lymphocyte apoptosis, but clinical features of ALPS were not present in the vast majority of these individuals. ALPS is a unique clinical syndrome in which in vitro abnormalities of lymphocyte apoptosis are associated with abnormal lymphoproliferation and autoimmunity. These findings provide evidence that apoptosis of activated lymphocytes is an important mechanism for maintaining immunologic homeostasis and self-tolerance in humans. Fas gene mutations account for impaired lymphocyte apoptosis in only a subset of patients with ALPS.

Tags: Female; Male

Descriptors: *Antigens, CD95--physiology--PH; *Apoptosis; *Autoimmune Diseases; *Lymphoproliferative Disorders; Anemia, Hemolytic, Autoimmune --etiology--ET; Animals; Antigens, CD95--genetics--GE; Autoimmune Diseases --complications--CO; Autoimmune Diseases--genetics--GE; Autoimmune Diseases--immunology--IM; Autoimmune Diseases--pathology--PA; Cells, Cultured; Child, Preschool; Follow-Up Studies; Humans; Hypergammaglobulinemia--etiology--ET; Infant; Lymphocyte Activation; Lymphoproliferative Disorders--complications--CO; Lymphoproliferative Disorders--genetics--GE; Lymphoproliferative Disorders--immunology--IM; Lymphoproliferative Disorders--pathology--PA; Mice; Neutropenia--etiology --ET; Pedigree; Receptors, Antigen, T-Cell, alpha-beta--analysis--AN; Splenomegaly--etiology--ET; Syndrome; T-Lymphocyte Subsets--immunology--IM; T-Lymphocyte Subsets--pathology--PA

CAS Registry No.: 0 (Antigens, CD95); 0 (Receptors, Antigen, T-Cell, alpha-beta)

Record Date Created: 19970317

Record Date Completed: 19970317

7/9/26

DIALOG(R)File 155:MEDLINE(R)

11765821 PMID: 8992969

Induction and prevention of colonic inflammation in IL-2-deficient mice.

Ehrhardt R O; Ludviksson B R; Gray B; Neurath M; **Strober W**

Mucosal Immunity Section, Laboratory of Clinical Investigation, National Institutes of Health, Bethesda, MD 20892, USA.

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Jan 15 **1997** , 158 (2) p566-73, ISSN 0022-1767 Journal Code: 2985117R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

Gene-targeted mice deficient for IL-2 (IL-2 -/- mice) are free of apparent disease when maintained under germfree conditions but develop colitis and autoimmunity in a conventional environment. Here we show that colitis can be reproducibly induced in IL-2 -/- mice, but not in IL-2 +/+ mice, by i.p. immunization with Ag in CFA; thus enabling the systematic study of the immunopathogenesis of the colitis. We found that TNP-KLH or TNP-OVA had the most significant effect in inducing colitis, and while TNP-KLH immunization leads to the early appearance of activated T cells in the colons of both IL-2 -/- and IL-2 +/+ mice, only lamina propria cells of IL-2 -/- mice produced high amounts of INF-gamma. Moreover, both infiltrating colon CD4+ (69%) and CD8+ (6%) T cells secrete large amounts of IFN-gamma; however, only the depletion of CD4+ T cells leads to abrogation of the inflammation. In further analysis, we showed that the high IFN-gamma production is IL-12 driven, since colonic tissues of IL-2 -/- mice but not IL-2 +/+ mice show the presence of heterodimeric IL-12 and co-administration of anti-IL-12 with TNP-KLH completely prevented colitis and significantly reduced IFN-gamma production. Finally, we demonstrate that IL-2 -/- mice are deficient in their ability to induce Th2 responses

after TNP-KLH challenge and that such immunization also leads to autoimmune-like phenomena in other organs of IL-2 -/- mice. These findings suggest that in the absence of IL-2 systemic administration of Ag induces primarily Th1 cells driven by overexpression of heterodimeric IL-12.

Descriptors: *Colitis--chemically induced--CI; *Colitis--prevention and control--PC; *Interleukin-2--deficiency--DF; *Interleukin-2--genetics--GE; *Trinitrobenzenes--immunology--IM; *Trinitrobenzenes--toxicity--TO; Animals; Antigens, T-Independent--immunology--IM; Colitis--immunology--IM; Haptens--immunology--IM; Hemocyanin--toxicity--TO; Immunization; Immunophenotyping; Interferon Type II--biosynthesis--BI; Interferon Type II--toxicity--TO; Interleukin-12--biosynthesis--BI; Interleukin-4--biosynthesis--BI; Intestinal Mucosa--metabolism--ME; Mice; Mice, Inbred C57BL; Mice, Mutant Strains; Mutagenesis, Site-Directed--genetics--GE; Ovalbumin--immunology--IM; Ovalbumin--toxicity--TO; T-Lymphocytes--metabolism--ME

CAS Registry No.: 0 (Antigens, T-Independent); 0 (Haptens); 0 (Interleukin-2); 0 (Trinitrobenzenes); 0 (trinitrophenyl keyhole limpet hemocyanin); 0 (trinitrophenyl-ovalbumin); 187348-17-0 (Interleukin-12); 207137-56-2 (Interleukin-4); 82115-62-6 (Interferon Type II); 9006-59-1 (Ovalbumin); 9013-72-3 (Hemocyanin)

Record Date Created: 19970211

Record Date Completed: 19970211

7/9/27

11762685 PMID: 8977180

Dysregulated intrathymic development in the IL-2-deficient mouse leads to colitis-inducing thymocytes.

Ludviksson B R; Gray B; **Strober W** ; Ehrhardt R O

Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

Journal of immunology (Baltimore, Md. - 1950) (UNITED STATES) Jan 1 **1997** , 158 (1) p104-11, ISSN 0022-1767 Journal Code: 2985117R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: AIM; INDEX MEDICUS

Gene-targeted mice lacking the IL-2 gene (IL-2 -/- mice) develop various forms of autoimmunity as well as severe colitis, either spontaneously in a conventional environment or after immunization with 2,4,6-trinitrophenol (TNP)-conjugated keyhole limpet hemocyanin (KLH) in a specific pathogen-free environment. We show here that the induction of colitis with TNP-KLH induces a change in the thymocyte population characterized by decreased numbers of double positive (DP; CD4+CD8+) thymocytes (IL-2 +/+, 45.2 x 10(6) vs IL-2 -/-, 23.6 x 10(6)) and increased numbers of single positive (SP; CD4+CD8- or CD4-CD8+) thymocytes (IL-2 +/+, 5.3 x 10(6) vs IL-2 -/-, 20.9 x 10(6)). The latter also bear activation markers. In addition, thymocytes from TNP-KLH-immunized IL-2 -/- mice produce more IFN-gamma and less IL-4 than similarly immunized IL-2 +/+ mice. These defects in thymocyte maturation and lymphokine production are IL-12 driven, since they are prevented when immunized IL-2 -/- mice are coadministered with anti-1L-12. Furthermore, we demonstrate that IL-2 -/- mice exhibit decreased cortical apoptosis as determined by thymocyte numbers and detection of apoptotic cells in situ. Finally, we show that colitis-inducing thymocytes are generated in the immunized IL-2 -/- thymus, since IL-2 +/+ mice develop colitis following injection of small numbers of single positive thymocytes from immunized IL-2 -/- mice but not from IL-2 +/+ mice. Taken together, these data indicate that, in the absence of IL-2, thymocyte maturation is abnormally directed by IL-12 toward the generation of mature, activated Th1-type thymocytes that are capable of mediating colitis.

Descriptors: *Colitis--etiology--ET; *Colitis--immunology--IM; *Interleu

kin-2--deficiency--DF; *Interleukin-2--genetics--GE; *Lymphocyte Activation--immunology--IM; *T-Lymphocytes--pathology--PA; *Thymus Gland--immunology--IM; *Thymus Gland--pathology--PA; Animals; Antigens, T-Independent--pharmacology--PD; Apoptosis--immunology--IM; Cell Differentiation--drug effects--DE; Cell Differentiation--immunology--IM; Colitis--pathology--PA; Hemocyanin--pharmacology--PD; Interleukin-12--pharmacology--PD; Mice; Mice, Mutant Strains; T-Lymphocytes--drug effects--DE; Thymus Gland--drug effects--DE

CAS Registry No.: 0 (Antigens, T-Independent); 0 (Interleukin-2); 0 (trinitrophenyl keyhole limpet hemocyanin); 187348-17-0 (Interleukin-12); 9013-72-3 (Hemocyanin)

Record Date Created: 19970130

Record Date Completed: 19970130

7/9/28

DIALOG(R)File 155:MEDLINE(R)

11542914 PMID: 8855736

Helicobacter pylori **infection and blood group antigens: lack of clinical association.**

Umlauft F; Keeffe E B; Offner F; Weiss G; Feichtinger H; **Lehmann E** ; Kilga-Nogler S; Schwab G; Propst A; Grussnewald K; Judmaier G

Department of Internal Medicine, University of Innsbruck, Austria.

American journal of gastroenterology (UNITED STATES) Oct **1996** , 91 (10) p2135-8, ISSN 0002-9270 Journal Code: 0421030

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

OBJECTIVES: Blood group antigens traditionally have been associated with a risk of developing peptic ulcer and gastric cancer. **Helicobacter pylori** is a bacterium associated with chronic active gastritis and ulcer disease, and its attachment to gastric mucosa was recently shown in vitro to be mediated by blood group Lewisb and H antigens. This study was designed to test the clinical relevance of this laboratory observation in patients undergoing endoscopy and gastric biopsy. METHODS: Blood group phenotypes and gastric biopsies for H. **pylori** and histology were determined and correlated in 384 patients undergoing upper endoscopy. Blood from healthy blood donors was tested for the same blood group antigens and used as a control group. RESULTS: The distribution of blood groups ABO, Lewis, Rhesus, and MN was similar among the patients undergoing endoscopy and a control group of 2369 healthy blood donors from the same geographic area. There was no correlation between H. **pylori** infection or the H. **pylori** -associated diseases, peptic ulcer or chronic active gastritis, with any blood group phenotype, including Lewisb, blood group O, or both. CONCLUSION: No in vivo correlation between H. **pylori** infection or disease and Lewisb or H antigen could be demonstrated. Moreover, patients with H. **pylori** infection and disease have a distribution of blood group antigens similar to a control population.

Tags: Female; Male

Descriptors: *Blood Group Antigens; * **Helicobacter** Infections--blood--BL; * **Helicobacter pylori** ; Biopsy; Case-Control Studies; Chi-Square Distribution; Cluster Analysis; Endoscopy, Gastrointestinal; Gastric Mucosa--pathology--PA; Gastritis--blood--BL; Gastritis--microbiology--MI; **Helicobacter** Infections--diagnosis--DI; **Helicobacter** Infections--epidemiology--EP; Humans; Logistic Models; Middle Aged; Peptic Ulcer--blood--BL; Peptic Ulcer--microbiology--MI

CAS Registry No.: 0 (Blood Group Antigens)

Record Date Created: 19961113

Record Date Completed: 19961113

7/9/29

used this model to evaluate the role of host responses in **Helicobacter** infections. BALB/c, C3H, and C57BL/6 mice were orally infected with a single strain of H. felis, and 2 and 11 weeks after infection, the mice were sacrificed and evaluated histologically for magnitude of H. felis infection. Intensity and extent of inflammation, and cellular composition of the inflammatory infiltrate. All three strains of mice demonstrated comparable levels of infection at 11 weeks, but the pattern and intensity of inflammation varied from minimal in BALB/c mice to severe in C57BL/6 mice. Gastric epithelial erosions were noted in C3H mice, and mucous cell hyperplasia was observed in C3H and C57BL/6 mice. Abundant mucosal mast cells were observed in the gastric tissues of all three mouse strains. Studies using major histocompatibility complex (MHC)-congenic mice revealed probable contributions by both MHC and non-MHC genes to **Helicobacter** -induced inflammation. Thus, large variations in the severity of disease were observed after infection of different inbred strains and congenic mice with a single isolate of H. felis. These results demonstrate the importance of the host response in disease outcome following gastric **Helicobacter** infection.

Tags: Comparative Study; Female; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Gastritis--etiology--ET; * **Helicobacter** Infections --complications--CO; *Major Histocompatibility Complex; Animals; Base Sequence; Chronic Disease; Disease Models, Animal; Disease Susceptibility; H-2 Antigens--genetics--GE; **Helicobacter** Infections--immunology--IM; Mice ; Mice, Inbred BALB C; Mice, Inbred C3H; Mice, Inbred C57BL; Molecular Sequence Data; Species Specificity; Specific Pathogen-Free Organisms; Stomach--microbiology--MI; Stomach--pathology--PA; Time Factors

CAS Registry No.: 0 (H-2 Antigens)

Record Date Created: 19960226

Record Date Completed: 19960226

?logoff hold

24feb05 12:20:33 User228206 Session D2367.2

$4.10 1.281 DialUnits File155

$6.51 31 Type(s) in Format 9

$6.51 31 Types

$10.61 Estimated cost File155

$0.26 TELNET

$10.87 Estimated cost this search

$10.87 Estimated total session cost 1.495 DialUnits

Status: Signed Off. (1 minutes)

Minor Descriptors: Adult; Aged; Cells, Cultured; Gastric Mucosa --immunology--IM; Middle Age; Monocytes--immunology--IM; Tissue Culture
CAS Registry No.: 0 (Tumor Necrosis Factor); 82115-62-6 (Interferon Type II)
Record Date Created: 19940309

17/9/9 (Item 9 from file: 144)
DIALOG(R)File 144:Pascal

13251181 PASCAL No.: 97-0521337
Differential stimulation of interleukin-12 (IL-12) and IL-10 by live and killed Helicobacter pylori in vitro and association of IL-12 production with gamma interferon-producing T cells in the human gastric mucosa
HAEBERLE H A; KUBIN M; BAMFORD K B; GARofalo R; GRAHAM D Y; EL-ZAATARI F; KARTTUNEN R; CROWE S E; REYES V E; ERNST P B
Department of Pediatrics a, University of Texas Medical Branch, Galveston , United States; The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania, United States; Department of Microbiology and Immunobiology, The Queens University of Belfast, Belfast, United Kingdom; Department of Medicine, Veterans Administration,, Houston, Texas, United States; Baylor College of Medicine, Houston, Texas, United States; Department of Medicine, University of Texas Medical Branch, Galveston, United States; Sealy Center for Molecular Sciences, University of Texas Medical Branch, Galveston, United States
Journal: Infection and immunity, 1997, 65 (10) 4229-4235
ISSN: 0019-9567 CODEN: INFIBR Availability: INIST-15757; 354000068337020360
No. of Refs.: 62 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: United States
Language: English
The objective of these experiments was to examine the ability of Helicobacter pylori to stimulate interleukin-10 (IL-10) or IL-12 and select for either Thl or Th2 cells. Gastric biopsy specimens were collected from patients who were categorized with respect to the presence of H. **pylori** and gastric disease as well as their age, gender, medications, and other factors. As Thl and Th2 cells are selected by IL-12 and IL-10, respectively, biopsy specimens were screened for mRNA and protein for these cytokines. Although mRNA for IL-12 and IL-10 was detected in biopsy specimens obtained from both infected and uninfected patients, IL-12 protein predominated. Levels of IL-10 and IL-12 in gastric tissue did not change in response to infection. Moreover, gamma **interferon** (IFN- gamma)-producing T cells were found in both the infected and the uninfected gastric mucosa. Stimulation of peripheral blood leukocytes from either infected or uninfected donors with various concentrations of live or killed H. **pylori** induced immunoreactive IL-12 and IL-10. After stimulation with live H. **pylori** , IL-12 levels increased more than 30-fold, whereas IL-10 levels increased only 2- to 5-fold, compared to cells stimulated with medium alone. Interestingly, killed H. **pylori** induced significantly more IL-10 ($P < 0.05$) than live H. **pylori** , while recombinant urease only induced IL-10. These results demonstrate that live H. pylori selectively stimulates the induction of IL-12 and Thl cells that produce IFN- gamma , whereas preparations used in oral vaccines induce more IL-10 and may favor Th2 cell responses.

English Descriptors: Helicobacter pylori; Host agent relation; Interleukin 12; Interleukin 10; Gamma interferon; T-Lymphocyte; In vitro; Stomach; Human
Broad Descriptors: Spirillaceae; Spirillales; Bacteria; Cytokine; Digestive system; Spirillaceae; Spirillales; Bacterie; Cytokine; Appareil digestif; Spirillaceae; Spirillales; Bacteria; Citoquina; Aparato digestivo

French Descriptors: Helicobacter pylori; Relation hote agent; Interleukine 12; Interleukine 10; **Interferon** gamma; Lymphocyte T; In vitro; Estomac; Homme

(R) Cited Ref Sci 1974-1989/Dec
(c) 1998 Inst for Sci Info
*File 434: Price change effective Jan 1, 2005. Enter HELP RATES 434 for details.
File 444:New England Journal of Med. 1985-2005/Feb W3
(c) 2005 Mass. Med. Soc.
File 467:ExtraMED(tm) 2000/Dec
(c) 2001 Informania Ltd.
*File 467: F467 no longer updates; see Help News467. 7.

Set	Items	Description

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Set	Items	Description
S1	4	'INTERFERON GAMMA (1-39)' OR 'INTERFERON GAMMA-1B' OR 'INTERFERON GAMMA-2A' OR 'INTERFERON GAMMA-4A'
S2	5	'INF GAMMA' OR 'INF- GAMMA'
S3	66	'INFG' OR 'INFGAMMA'
S4	1	'GAMMA INF'
S5	76	S1 OR S2 OR S3 OR S4
S6	1941	INF
S7	20388	E1-E12
S8	188	E13-E24
S9	20397	HELICOBACTER?
S10	20397	S7 OR S8 OR S9
S11	1	S5 AND S10
S12	698425	INTERFERON?
S13	158239	PYLORI OR HELICOBACT?
S14	1162	S12 (100N) S13
S15	839	S14/1999:2005
S16	323	S14 NOT S15
S17	50	TARGET - S16

?t s17/9/16 17 24 26 27 28 30 38

17/9/16 (Item 16 from file: 144)
DIALOG(R)File 144:Pascal

11983357 PASCAL No.: 95-0169701
Gastric juice neopterin in Helicobacter pylori infection : Pathogenesis and host response in Helicobacter pylori infection
MELICHAR B; MALIROVA E; BURES J; KOMARKOVA O; KOLESAR J; REJCHRT S; FIXA B
Charles univ. medical school, second dep. internal medicine, 50036 Hradec Kralove, CZE
Meeting on pathogenesis and host response in Helicobacter pylori infection (Helsingoer DNK) 1994-06
Journal: FEMS immunol. med. microbiol., 1995, 10 (3-4) 335-338
ISSN: 0928-8244 Availability: INIST-17567B; 354000059688550220
No. of Refs.: 21 ref.
Document Type: P (Serial); C (Conference Proceedings) ; A (Analytic)
Country of Publication: Netherlands
Language: English
Neopterin, a pteridine compound produced by macrophages activated by **interferon** -gamma, is widely used to assess the activation of cellular immunity. An elevation in serum or urinary neopterin reflects immune activation in many different disorders, including viral infections, cancer, autoimmune diseases or acute myocardial infarction, but less attention has been paid to neopterin concentration in other biological fluids. The aim of the present study was to examine neopterin concentration in gastric juice. An association with the presence of **Helicobacter pylori** , a bacterium linked to the most common disorders of upper digestive tract, was also investigated. Gastric juice was obtained at endoscopy from 61 patients.Neopterin was determined by a radioimmunoassay and the presence of H. pylori was examined by urease test. The macroscopic finding of bile in

gastric juice was associated with significantly higher neopterin levels compared to patients where no bile was noted (15.5 +- 15.6 vs. 2.1 +- 3.0 nmol/l, P<0.001). However, similar concentrations were observed in the H. pylori positive and H. pylori negative patients (7.6 +- 12.0 vs. 11.1 +- 14.9 nmol/l). Even in the absence of macroscopic bile contamination, no significant difference could be found between the infected and uninfected patients (2.3 +- 3.2 vs. 1.3 +- 1.9 nmol/l), and the patients with duodenal ulcer and normal findings (3.8 +- 4.6 vs. 1.6 +- 1.9 nmol/l). The contamination of gastric juice with bile represents the limitation for the use of neopterin as a marker of immune activation in the gastric mucosa. Rather than an index of immune activation, gastric juice neopterin concentration represents a marker of duodenogastric reflux

English Descriptors: Helicobacter pylori; Human; Macrophage; Gamma **interferon** ; Cellular immunity; Immunostimulation; Defense; Gastric juice ; Biological marker; Pathogenesis; Gastritis
Broad Descriptors: Spirillaceae; Spirillales; Bacteria; Digestive diseases; Gastric disease; Spirillaceae; Spirillales; Bacterie; Appareil digestif pathologie; Estomac pathologie; Spirillaceae; Spirillales; Bacteria; Aparato digestivo patologia; Estomago patologia

French Descriptors: **Helicobacter pylori** ; Homme; Macrophage; **Interferon** gamma; Immunite cellulaire; Immunostimulation; Defense organisme; Pteridine; Liquide gastrique; Marqueur biologique; Pathogenie; Gastrite; Neopterine

Classification Codes: 002B05B02F

17/9/17 (Item 17 from file: 144)
DIALOG(R)File 144:Pascal

11113608 PASCAL No.: 93-0620632
Contact of lymphocytes with Helicobacter pylori augments natural killer cell activity and induces production of gamma interferon
TARKKANEN J; KOSUNEN T U; SAKSELA E
Univ. Helsinki, dep. pathology, 00290 Helsinki, Finland
Journal: Infection and immunity, 1993, 61 (7) 3012-3016
ISSN: 0019-9567 CODEN: INFIBR Availability: INIST-15757; 354000034705730380
No. of Refs.: 56 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: USA
Language: English
We studied the capacity of glutaraldehyde-fixed Helicobacter pylori to stimulate natural killer (NK) cell activity. Bacteria were incubated overnight with peripheral blood lymphocytes enriched for large granular lymphocytes (LGL), the mediators of non-major histocompatibility complex-restricted cellular cytotoxicity. Then, the cytolytic activity of LGL was tested against various tumor target cells. We observed that efficient cytolytic activity was generated against resistant and nonresistant tumor target cell lines. Nine local clinical isolates of H. pylori and the reference strain NCTC 11637 were tested, and they all were equally effective in inducing NK cell activity

English Descriptors: **Helicobacter pylori** ; Cell cell interaction; Lymphocyte; Immunostimulation; Natural killer cell; Secretion; Gamma **interferon** ; Cytotoxicity; Cell culture
Broad Descriptors: Spirillaceae; Spirillales; Bacteria; Cytokine; Spirillaceae; Spirillales; Bacterie; Cytokine; Spirillaceae; Spirillales; Bacteria; Citoquina

French Descriptors: **Helicobacter pylori** ; Interaction cellulaire; Lymphocyte; Immunostimulation; Cellule NK; Secretion; **Interferon** gamma; Cytotoxicite; Culture cellulaire

Classification Codes: 002A06C05B

17/9/24 (Item 24 from file: 144)
DIALOG(R)File 144:Pascal
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12863350 PASCAL No.: 97-0122207
Expression of mRNA for interferon-gamma, interleukin-10, and interleukin-12 (p40) in normal gastric mucosa and in mucosa infected with Helicobacter pylori
KARTTUNEN R A; KARTTUNEN T J; YOUSFI M M; EL-ZIMAITY H M T; GRAHAM D Y; EL-ZAATARI F A K
Dept. of Medicine, Veterans Affairs Medical Center, Houston, Texas, United States; Inflammatory Bowel Disease Laboratory, Veterans Affairs Medical Center, Houston, Texas, United States; Division of Molecular Virology, Baylor College of Medicine, Houston, Texas, United States
Journal: Scandinavian journal of gastroenterology, 1997, 32 (1) 22-27
ISSN: 0036-5521 CODEN: SJGRA4 Availability: INIST-12513; 354000061379300040
No. of Refs.: 46 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Norway
Language: English
Background : We studied the mRNA expressions of interferon-gamma (IFN-gamma), interleukin-10 (IL-10), and IL-12 in gastric biopsy and blood samples from patients with and without Helicobacter pylori infection, by reverse-transcription polymerase chain reaction (RT-PCR). Methods : RT-PCR was performed on total RNA preparations, and the expressed mRNA were semiquantitated on the basis of band intensities on Southern blots. Results : In gastric mucosa the expression of IFN- gamma and IL-10 was found in most patients with and without H. pylori infection, whereas IL-12 was found in most of the infected ones. The level of IFN- gamma and IL-10 did not differ between groups, whereas the IL-12 level was significantly higher in those with H. pylori infection. In the blood IFN- gamma expression was found in most samples, with higher level in patients with gastritis than in normals. Few blood samples (33%) had IL-12, and none had IL-10. Conclusion : IFN- gamma and IL-10 expressions in healthy mucosa may indicate a biologic role in a healthy state. IL-12 expression in mucosa was related to the presence of bacterial stimulant and therefore resembles proinflammatory cytokines.

English Descriptors: Gastritis; Campylobacter infection; Helicobacter pylori; Gene expression; Messenger RNA; Gamma interferon; Interleukin 10; Interleukin 12; Mucosa; Stomach; Cytokine; Comparative study; Human
Broad Descriptors: Bacteriosis; Infection; Spirillaceae; Spirillales; Bacteria; Digestive diseases; Gastric disease; Bacteriose; Infection; Spirillaceae; Spirillales; Bacterie; Appareil digestif pathologie; Estomac pathologie; Bacteriosis; Infeccion; Spirillaceae; Spirillales; Bacteria; Aparato digestivo patologia; Estomago patologia
French Descriptors: Gastrite; Campylobacteriose; **Helicobacter** pylori; Expression genique; RNA messager; Interferon gamma; Interleukine 10; Interleukine 12; Muqueuse; Estomac; Cytokine; Etude comparative; Homme

Classification Codes: 002B05B02F

17/9/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11555037 PMID: 8867497

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*File 370: This file is closed (no updates). Use File 47 for more current information.
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*File 399: Use is subject to the terms of your user/customer agreement. Alert feature enhanced for multiple files, etc. See HELP ALERT.
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
(c) 1998 Inst for Sci Info
*File 434: Price change effective Jan 1, 2005. Enter HELP RATES 434 for details.
File 444:New England Journal of Med. 1985-2005/Feb W3
(c) 2005 Mass. Med. Soc.
File 467:ExtraMED(tm) 2000/Dec
(c) 2001 Informania Ltd.
*File 467: F467 no longer updates; see Help News467. 7.

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S9	20397	HELICOBACTER?
S10	20397	S7 OR S8 OR S9
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S13	158239	PYLORI OR HELICOBACT?
S14	1162	S12 (100N) S13
S15	839	S14/1999:2005
S16	323	S14 NOT S15
S17	50	TARGET - S16

?t s17/9/6 8 9 10

17/9/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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10754849 PMID: 7959191
Gastric T lymphocyte responses to Helicobacter pylori in patients with H pylori colonisation.
Fan X J; Chua A; Shahi C N; McDevitt J; Keeling P W; Kelleher D
Department of Clinical Medicine, St James's Hospital, Trinity College, Dublin, Ireland.
Gut (ENGLAND) Oct 1994, 35 (10) p1379-84, ISSN 0017-5749
Journal Code: 2985108R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS
Helicobacter pylori has been identified as a dominant factor in the pathogenesis of duodenal ulcer. The aim of this study was to examine peripheral blood and gastric lymphocyte proliferation and cytokine production in patients with H pylori colonisation. Sixty five dyspeptic patients attending for endoscopy were studied; 35 of these were H pylori positive and 30 H pylori negative as assessed by culture, histology, and

Enhanced T-helper 2 lymphocyte responses: immune mechanism of Helicobacter pylori infection.

Fan X G; Yakoob J; Fan X J; Keeling P W
Department of Clinical Medicine, St. James's Hospital, Dublin.

Irish journal of medical science (IRELAND) Jan-Mar 1996, 165 (1) p37-9, ISSN 0021-1265 Journal Code: 7806864
Publishing Model Print
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Several lines of evidence implicate **Helicobacter pylori** (H. **pylori**) infection in gastroduodenal inflammation. However, the exact pathogenesis of H. **pylori** infection is not fully understood. T-helper (TH) lymphocytes may be subdivided into TH1 and TH2 cells based on the distinct patterns of cytokine production. TH1 reaction is associated with immunity or resistance to infection, while TH2 reaction is associated with the progression or persistence of infection. The production of **interferon** -gamma (INF-gamma) and interleukin 2 (IL-2), which are type 1 cytokines, is decreased in H. **pylori** infection. Enhanced production of type 2 cytokines (IL-4) and IL-6) is observed in individuals with H. **pylori** infection. Suppressed proliferative responses of peripheral blood and gastric lymphocytes have also been demonstrated in patients with H. **pylori** colonisation, suggesting that specific T-cell responses may be down-regulated by an enhanced TH2 reaction. Suppressed TH1 and enhanced TH2 responses in H. **pylori** infection may be involved in the immunopathogenesis of chronic H. **pylori** infection. (30 Refs.)

Descriptors: *Gastrointestinal Diseases--microbiology--MI; *Helicobacter Infections--immunology--IM; *Helicobacter pylori--immunology--IM; *T-Lympho cytes, Helper-Inducer--metabolism--ME; Chronic Disease; Gastrointestinal Diseases--immunology--IM; Gastrointestinal Diseases--physiopathology--PP; Helicobacter Infections--physiopathology--PP; Humans; Interferon-alpha --biosynthesis--BI; Interleukin-2--biosynthesis--BI
CAS Registry No.: 0 (Interferon-alpha); 0 (Interleukin-2)
Record Date Created: 19961203
Record Date Completed: 19961203

17/9/27 (Item 27 from file: 155)

10915420 PMID: 7698689

Interferon gamma and interleukin 4 secreting cells in the gastric antrum in Helicobacter pylori positive and negative gastritis.

Karttunen R; Karttunen T; Ekre H P; MacDonald T T
Department of Paediatric Gastroenterology, St Bartholomew's Hospital, London.

Gut (ENGLAND) Mar 1995, 36 (3) p341-5, ISSN 0017-5749 Journal Code: 2985108R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS

Little is known of the function of the T cells in the inflammatory infiltrate in **Helicobacter** pylori associated gastritis. This study thus measured T cell in vivo activation by enumerating the frequency of interferon gamma (IFN gamma) and interleukin 4 (IL 4) secreting cells isolated from the gastric antral mucosa in patients with or without gastritis and in H pylori positive and negative gastritis. Fifty four samples were examined for cytokine secretion. Four antral biopsy specimens from each patient (n = 51) were taken during diagnostic endoscopy. One was used to estimate histological gastritis and the presence of H pylori, and three of the samples were used to isolate T cells by enzymatic digestion.

IFN gamma and IL 4 secreting cells were enumerated by ELISPOT. Thirty four samples had gastritis and 79% of those were H pylori positive. None of the samples from non-inflamed mucosa had H pylori. The numbers of IFN gamma secreting cells per 10(5) T cells were higher in gastritis than in normal mucosa (145 v 20 IFN gamma spots, p < 0.01), and higher in H pylori negative than H pylori positive gastritis (371 v 110 IFN gamma spots, p < 0.05). The frequencies of IL 4 secreting cells did not differ between gastritis and non-inflamed mucosa. In conclusion, there is an increase in IFN gamma secreting cells but not in IL 4 secreting cells in H pylori positive and negative gastritis. It is not known if this TH1 type reaction has a pathogenetic or protective role.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Gastritis--pathology--PA; *Helicobacter Infections --pathology--PA; *Helicobacter **pylori** ; *Interferon Type II--secretion--SE ; *Interleukin-4--secretion--SE; *Pyloric Antrum--secretion--SE; Adult; Aged; Aged, 80 and over; Cell Division; Gastritis--immunology--IM; Helicobacter Infections--immunology--IM; Humans; Lymphocyte Count; Middle Aged; Pyloric Antrum--pathology--PA; T-Lymphocytes--pathology--PA

CAS Registry No.: 207137-56-2 (Interleukin-4); 82115-62-6 (Interferon Type II)

Record Date Created: 19950504

Record Date Completed: 19950504

17/9/28 (Item 28 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05402046 Genuine Article#: VW292 Number of References: 52

Title: HELICOBACTER-PYLORI STIMULATES INDUCIBLE NITRIC-OXIDE SYNTHASE EXPRESSION AND ACTIVITY IN A MURINE MACROPHAGE CELL-LINE

Author(s): WILSON KT; RAMANUJAM KS; MOBLEY HLT; MUSSELMAN RF; JAMES SP; MELTZER SJ

Corporate Source: UNIV MARYLAND HOSP,DIV GASTROENTEROL,SCH MED,ROOM N3W62,22 S GREENE ST/BALTIMORE//MD/21210; UNIV MARYLAND,SCH MED,DEPT MED,DIV GASTROENTEROL/BALTIMORE//MD/21201; UNIV MARYLAND,SCH MED,DEPT MED,DIV INFECT DIS/BALTIMORE//MD/21201; BALTIMORE VET AFFAIRS MED CTR/BALTIMORE//MD/00000

Journal: GASTROENTEROLOGY, 1996, V111, N6 (DEC), P1524-1533

ISSN: 0016-5085

Language: ENGLISH Document Type: ARTICLE

Geographic Location: USA

Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences; CC CLIN--Current Contents, Clinical Medicine

Journal Subject Category: GASTROENTEROLOGY AND HEPATOLOGY

Abstract: Background & Aims: Helicobacter pylori uniquely colonizes the human stomach and produces gastric mucosal inflammation, High-output nitric oxide production by inducible nitric oxide synthase (iNOS) is associated with immune activation and tissue injury, Because mononuclear cells comprise a major part of the cellular inflammatory response to H. pylori infection, the ability of H. **pylori** to induce iNOS in macrophages was assessed, Methods: H. **pylori** preparations were added to RAW 264.7 murine macrophages, and iNOS expression was assessed by Northern blot analysis, enzyme activity assay, and NO2- release, Results: Both whole H. **pylori** and French press lysates induced concentration-dependent NO2- production, with peak levels 20 fold above control. These findings were paralleled by marked increases in iNOS messenger RNA and enzyme activity levels, iNOS expression was synergistically increased with **interferon** gamma, indicating that the H. **pylori** effect can be amplified by other macrophage-activating factors, Studies of lipopolysaccharide (LPS) content and polymyxin B inhibition of LPS suggested that the H. **pylori** effect was attributable to both LPS-dependent and -independent mechanisms, Conclusions: iNOS expression in macrophages is activated by highly stable H. **pylori** products and may play an important role in the pathogenesis of H. **pylori** -associated gastric mucosal disease.

Identifiers--KeyWords Plus: MONOCYTE-DERIVED MACROPHAGES; HUMAN MONONUCLEAR

PHAGOCYTES; INTERFERON-GAMMA; PEPTIC-ULCERATION; GASTRIC-CARCINOMA; MOUSE MACROPHAGES; SURFACE-PROTEINS; LIPOPOLYSACCHARIDE; INFECTION; INDUCTION

Research Fronts: 94-1492 003 (HELICOBACTER-PYLORI INFECTION; IMPLICATIONS FOR ULCER THERAPY; ACID-PEPTIC DISEASE)

94-7582 002 (NITRIC-OXIDE IN CARCINOGENESIS; HUMAN P53 GENE; INHIBITION OF THE ZINC FINGER-TYPE YEAST TRANSCRIPTION ACTIVATOR LAC9)

94-0394 001 (NITRIC-OXIDE SYNTHASE; NADPH-DIAPHORASE-POSITIVE MYENTERIC NEURONS OF THE RAT ILEUM; FUNCTIONAL EXPRESSION)

94-0733 001 (O-SPECIFIC POLYSACCHARIDE; CAPSULAR ANTIGEN; STRUCTURAL ELUCIDATION)

94-2143 001 (NITRIC-OXIDE SYNTHASE; PEROXYNITRITE DECOMPOSITION; LUNG ALVEOLAR INJURY; FREE-RADICAL CHEMISTRY)

94-3070 001 (RAT SKELETAL-MUSCLE; DEVELOPMENTAL REGULATION; YEAST SACCHAROMYCES-CEREVISIAE)

94-4768 001 (NITRIC-OXIDE SYNTHASE; ANTIMICROBIAL ARMATURE OF HUMAN MACROPHAGES; REACTIVE NITROGEN INTERMEDIATES)

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MILLER MJS, 1995, V109, P4175, GASTROENTEROLOGY
MILLER MJS, 1993, V264, P11, J PHARMACOL EXP THER
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17/9/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11535832 PMID: 8844467
Cytokine gene expression in the gastric mucosa: its role in chronic gastritis.
Ishihara S; Fukuda R; Fukumoto S
Second Department of Internal Medicine, Shimane Medical University, Japan.
Journal of gastroenterology (JAPAN) Aug 1996, 31 (4) p485-90, ISSN 0944-1174 Journal Code: 9430794
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
There have been few studies of cytokine expression in the gastric mucosa of patients with chronic gastritis. In the present study, to elucidate the expression of cytokines in the gastric mucosa and the immunopathological roles played by these cytokines in chronic gastritis, we investigated cytokine gene expression, by reverse transcription polymerase chain reaction, in gastric biopsy specimens obtained from 29 endoscopically normal patients with chronic gastritis. The cytokines examined and the mRNA positivity were: interleukin (IL)-1 beta (21%), IL-2 (0%), IL-3 (7%), IL-4 (41%), IL-5 (17%), IL-6 (53%), IL-8 (98%), **interferon** gamma (IFN-gamma) (69%), and tumor necrosis factor alpha (TNF-alpha) (24%). Although the histological severity of the gastritis was closely associated with **Helicobacter pylori** infection, the positivities of these cytokine mRNAs did not show a relationship with either H. **pylori** infection or with histological inflammation. Our findings suggest that the gastric mucosa responds to all exogenous antigens, including H. **pylori** , in the same fashion immunologically, and that these cytokines do not contribute to the induction of inflammation associated with H. **pylori** infection.
Tags: Female; Male
Descriptors: *Gastric Mucosa--metabolism--ME; *Gastritis--genetics--GE; *Interferon Type II--biosynthesis--BI; *Interleukins--biosynthesis--BI; *Tumor Necrosis Factor-alpha--biosynthesis--BI; Gastritis--metabolism--ME; Gastritis--microbiology--MI; Gene Expression; Helicobacter Infections --diagnosis--DI; **Helicobacter** Infections--metabolism--ME; **Helicobacter pylori** --isolation and purification--IP; Humans; **Interferon** Type II --genetics--GE; Interleukins--genetics--GE; Middle Aged; Polymerase Chain Reaction; RNA, Messenger--genetics--GE; Tumor Necrosis Factor-alpha --genetics--GE
CAS Registry No.: 0 (Interleukins); 0 (RNA, Messenger); 0 (Tumor Necrosis Factor-alpha); 82115-62-6 (Interferon Type II)
Record Date Created: 19961223
Record Date Completed: 19961223

17/9/38 (Item 38 from file: 159)
DIALOG(R)File 159:Cancerlit

02284871 97001475 PMID: 8844467
Cytokine gene expression in the gastric mucosa: its role in chronic gastritis.
Ishihara S; Fukuda R; Fukumoto S
Second Department of Internal Medicine, Shimane Medical University, Japan.
J Gastroenterol (JAPAN) Aug 1996, 31 (4) p485-90, ISSN 0944-1174

Journal Code: 9430794
Document Type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Subfile: INDEX MEDICUS

There have been few studies of cytokine expression in the gastric mucosa of patients with chronic gastritis. In the present study, to elucidate the expression of cytokines in the gastric mucosa and the immunopathological roles played by these cytokines in chronic gastritis, we investigated cytokine gene expression, by reverse transcription polymerase chain reaction, in gastric biopsy specimens obtained from 29 endoscopically normal patients with chronic gastritis. The cytokines examined and the mRNA positivity were: interleukin (IL)-1 beta (21%), IL-2 (0%), IL-3 (7%), IL-4 (41%), IL-5 (17%), IL-6 (53%), IL-8 (98%), **interferon** gamma (IFN-gamma) (69%), and tumor necrosis factor alpha (TNF-alpha) (24%). Although the histological severity of the gastritis was closely associated with **Helicobacter pylori** infection, the positivities of these cytokine mRNAs did not show a relationship with either H. **pylori** infection or with histological inflammation. Our findings suggest that the gastric mucosa responds to all exogenous antigens, including H. **pylori** , in the same fashion immunologically, and that these cytokines do not contribute to the induction of inflammation associated with H. **pylori** infection.

Tags: Female; Human; Male
Major Descriptors: *Gastric Mucosa--metabolism--ME; *Gastritis--genetics --GE; *Interferon Type II--biosynthesis--BI; *Interleukins--biosynthesis --BI; *Tumor Necrosis Factor--biosynthesis--BI
Minor Descriptors: Gastritis--metabolism--ME; Gastritis--microbiology --MI; Gene Expression; Helicobacter Infections--diagnosis--DI; **Helicobacter** Infections--metabolism--ME; **Helicobacter pylori** --isolation and purification--IP; **Interferon** Type II--genetics--GE; Interleukins--genetics--GE; Middle Age; Polymerase Chain Reaction; RNA, Messenger--genetics--GE; Tumor Necrosis Factor--genetics--GE
CAS Registry No.: 0 (Interleukins); 0 (RNA, Messenger); 0 (Tumor Necrosis Factor); 82115-62-6 (Interferon Type II)
Record Date Created: 19961223

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    $0.06 Estimated cost File149
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          $0.08    0.026 DialUnits File159
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    $0.26 TELNET
   $15.35 Estimated cost this search
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### Status: Signed Off. (1 minutes)
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Second-Generation--therapeutic use--TU; *Fluoxetine--therapeutic use--TU; *Serotonin Uptake Inhibitors--therapeutic use--TU; Adolescent; Adult; Humans; Prospective Studies
CAS Registry No.: 0 (Antidepressive Agents, Second-Generation); 0 (Serotonin Uptake Inhibitors); 54910-89-3 (Fluoxetine)
Record Date Created: 19980514
Record Date Completed: 19980514

7/9/8
DIALOG(R)File 155:MEDLINE(R)

12208072 PMID: 9515678
Nested primers improve sensitivity in the detection of Helicobacter pylori **by the polymerase chain reaction.**
Bamford K B ; Lutton D A; O'Loughlin B; Coulter W A; Collins J S
Department of Microbiology and Immunobiology, The Queen's University of Belfast, UK.
Journal of infection (ENGLAND) Jan **1998** , 36 (1) p105-10, ISSN 0163-4453 Journal Code: 7908424
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
To investigate potential routes of spread of infection by the polymerase chain reaction (PCR) it is important that the technique is effective in the types of specimen to be investigated. To establish the limits of detection of **Helicobacter pylori** by PCR in clinical material from the gastric mucosa, faeces, dental plaque and oral rinses, samples were seeded with known numbers of bacteria. DNA extraction was followed by amplification with primers from the urease C gene. Nested primers were used to amplify the PCR product which was detected using a digoxigenin-labelled probe. Faeces or plaque inhibited the single reaction 10(2)-10(6) fold. A second amplification using nested primers and probing increased the sensitivity to a level similar to that obtained with pure culture. This method is potentially useful with less likelihood of false negative results when trying to detect H. **pylori** by PCR in highly contaminated, clinical material.
Tags: Research Support, Non-U.S. Gov't
Descriptors: ***Helicobacte** r **pylori** --isolation and purification--IP; *Polymerase Chain Reaction--methods--MT; DNA Primers; Dental Plaque --microbiology--MI; Face--microbiology--MI; Gastric Mucosa--microbiology --MI; **Helicobacter** Infections--etiology--ET; Humans; Sensitivity and Specificity
CAS Registry No.: 0 (DNA Primers)
Record Date Created: 19980428
Record Date Completed: 19980428

7/9/9
DIALOG(R)File 155:MEDLINE(R)

12190439 PMID: 9496938
Lymphocytes in the human gastric mucosa during Helicobacter pylori **have a T helper cell 1 phenotype.**
Bamford K B ; Fan X; Crowe S E; Leary J F; Gourley W K; Luthra G K; Brooks E G; Graham D Y; Reyes V E; Ernst P B
Department of Microbiology and Immunology, The Queen's University of Belfast, Northern Ireland.
Gastroenterology (UNITED STATES) Mar **1998** , 114 (3) p482-92, ISSN 0016-5085 Journal Code: 0374630
Contract/Grant No.: CHD 35741; HD; NICHD; DK 50669; DK; NIDDK; DK 51577; DK; NIDDK

Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS; AIDS/HIV

BACKGROUND & AIMS: Studies have shown that gastric T cells are increased during **Helicobacter pylori** infection. The purpose of this study was to characterize the human gastric T-cell responses in the presence or absence of H. **pylori** . METHODS: T-cell surface antigens were examined by immunohistochemistry or after isolation for evaluation of surface antigens and cytoplasmic cytokines using flow cytometry. RESULTS: CD4+ and CD8+ T cells were increased in situ during infection with H. **pylori** . Freshly isolated gastric T cells expressed cytoplasmic interferon gamma (IFN-gamma) and interleukin (IL)-2 after a brief stimulation. Simultaneous four-color flow cytometry demonstrated that both CD8+ and CD4+ T cells expressed IFN-gamma. Because stimulation through CD30 favors the induction of IL-5 and Th2 cells, gastric and colonic T cells were examined for CD30 expression. Consistent with the notion that Th2 cells are found in the intestine, CD30 was evident throughout the lamina propria of the colon but was virtually absent in the stomach. Furthermore, freshly isolated gastric T cells produced little IL-4 and virtually no IL-5 or tumor necrosis factor beta. CONCLUSIONS: These observations show that gastric T cells resemble the Th1 type, which may explain their failure to induce immunity to H. **pylori** and their ability to contribute to the pathogenesis of gastric disease.

Tags: Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Gastric Mucosa--immunology--IM; * **Helicobacter** Infections --immunology--IM; * **Helicobacter pylori** ; *Th1 Cells--physiology--PH; Adult; Antigens, CD30--analysis--AN; Cells, Cultured; Humans; Interferon Type II--biosynthesis--BI; Middle Aged

CAS Registry No.: 0 (Antigens, CD30); 82115-62-6 (Interferon Type II)

Record Date Created: 19980319
Record Date Completed: 19980319

7/9/10

DIALOG(R)File 155:MEDLINE(R)

12093345 PMID: 9391738

The incidence of acute and remote seizures in children with intraventricular hemorrhage.

Strober J B ; Bienkowski R S; Maytal J

Division of Pediatric Neurology and Pediatric Research Center, Schneider Children's Hospital, Long Island Jewish Medical Center, Albert Einstein College of Medicine, New Hyde Park, NY, USA.

Clinical pediatrics (UNITED STATES) Nov **1997** , 36 (11) p643-7, ISSN 0009-9228 Journal Code: 0372606

Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: AIM; INDEX MEDICUS

Seizures are a well-known complication of intraventricular hemorrhage (IVH) in premature infants; however, the rate at which they occur is not known. The authors decided, therefore, to investigate both the incidence of acute and remote seizures in infants with IVH and the association with the grade of hemorrhage. One hundred and three infants with IVH were identified and their records were reviewed for acute seizures, remote seizures, and associated morbidity and mortality. The average gestational age of these infants was 29 weeks (range, 23-40 weeks). Of the 103 infants, 32 (31%) developed grade 4 IVH; 19 (18%), grade 3 IVH; and 52 (50%), grades 1 and 2 IVH. Seventeen (17%) patients had acute seizures during their first month of life. Six of the 61 patients (10%) who survived the neonatal period and